The Response of Fodderbeet to Salinity

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2007

RESPONSE OF FODDERBEET TO SALINITY

Introduction of a non-conventional fodder crop (Fodderbeet) to salt affected lands of Pakistan

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The Response of Fodderbeet to Salinity: Introduction of a non-conventional fodder crop (Fodderbeet) to salt affected lands of Pakistan
Ph D-thesis Vrije Universiteit, Amsterdam

ISBN: 978-969-409-188-4

Front page designing: Muhammad Jawad
Composing and conversion to Coral Draw: Muhammad Jawad

Thesis 2007-1 of the Department of System Ecology, Institute of Ecological Science, Faculty of Earth and Life Sciences, Vrije Universiteit, Amsterdam

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VRIJE UNIVERSITEIT

The Response of Fodderbeet to Salinity

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan

de Vrije Universiteit Amsterdam,

op gezag van de rector magnificus

prof.dr. L.M. Bouter,

in het openbaar te verdedigen

ten overstaan van de promotiecommissie

van de faculteit der Aard- en Levenswetenschappen

op donderdag 22 maart 2007 om 13.45 uur

in de aula van de universiteit,

De Boelelaan 1105

door

Banaras Hussain Niazi

geboren te Rawalpindi, Pakistan

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Dedicated to my parents (Late) who always prayed for all success in my life

FOREWORD

I am highly grateful to Almighty Allah who has favored me the most and blessed me with His kindness and mercifulness. All the encouragement for completion of my research for a humble cause of science and humanity has been provided to me by His grace. Sometimes an event of life inspires to initiate for some new things. It happened to me when I met Dr. Jelte Rozema at the US-Pakistan Biosaline Research Workshop held at Karachi, Pakistan in 1985. We discussed the possible avenues of collaborative research and agreed upon exploration of feasibility of fodderbeet cultivation and its promotion in Pakistan. Of course I am thankful to Dr. Zaib-un-Nisa Abdullah through whom that meeting was arranged. I started my research work and exchanged our research results. It further encouraged me to continue my work. During 1990-1991, I visited the Free University, Amsterdam, The Netherlands and was exposed to some more sophisticated research work in the relevant field. At the same time Dr. Jelte Rozema was kind enough to offer me an opportunity to complete my research data for the promotion of my Ph. D. Although it took a longer period for maturation of the project, but I still feel myself lucky that the present manuscript has been recommended for Ph. D. promotion. I am highly obliged to Prof Drs. Rien Aerts for his sincere support and encouragement in the promotion of this thesis. I am highly grateful to Dr. Bert De Boer for sparing his precious time for valuable technical comments for improvement of the thesis.

I am thankful to my friends from The Netherlands as well as Pakistan who encouraged me and provided their valuable consultation and help, especially, Drs. Huub Van der Erve, Drs. Rinus Otte, Mr. Rob Broekman in The Netherlands and Dr. M. Salim, and other colleagues in Pakistan. The Centre for Development Cooperation Services (CDCS) and The Netherlands Organization for International Cooperation in Higher Education (NUFFIC) played a vital role in providing the financial help during my stay in The Netherlands. I appreciate its helpful activities not for myself but for all other research departments who are getting help from it. I am very grateful to my family, who encouraged me a lot and patiently waited for the promotion of my Ph. D degree.

I pray to Almighty Allah to prove this research work for the progress of my country. It is a fact that there is no end to research in any field. In the present case, there is still a need of further investigation to convince our farmers for adopting this non-conventional fodder crop for their fields. Production of seed for cultivation of the fodderbeet at commercial scale is another issue still to resolve.

I wish this thesis to be a milestone for further collaboration in Dutch-Pakistan Cooperation Research Project.

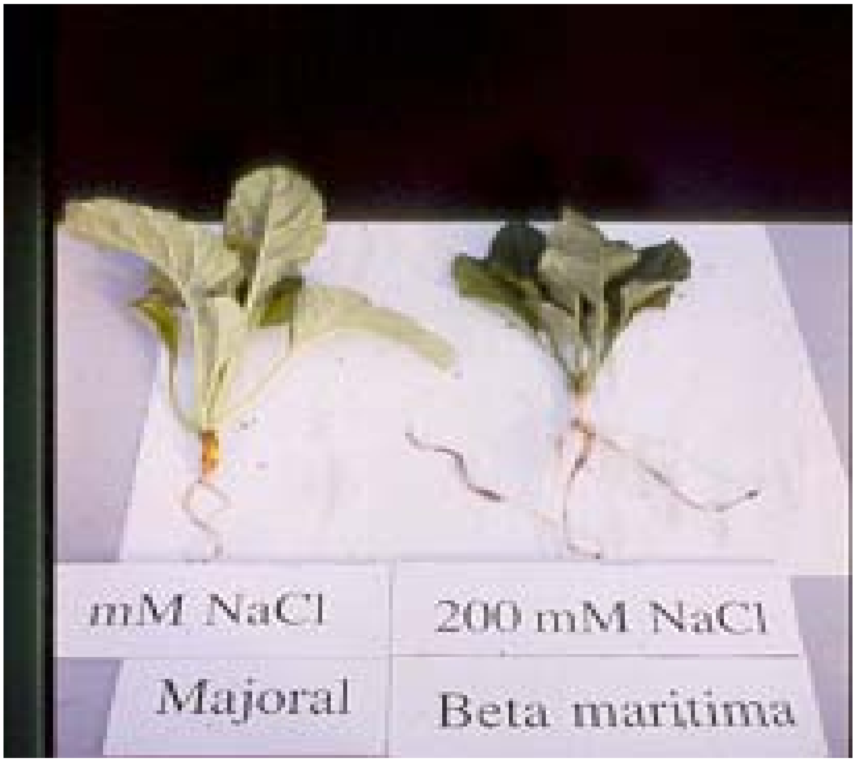
Banaras H. Niazi

Contents

List of abbreviations used in the thesis

AEARC	Atomic Energy Agriculture Research Centre
AZRSS	Arid Zone Research Sub-station
CDCS	Centre for Development Cooperation Services
DRIP	Drainage Research Institute of Pakistan
FYM	Farm Yard Manure
GOP	Government of Pakistan
IWASRI	International Waterlogging and Salinity Research Institute
LAR	Leaf Area Ratio
LWR	Leaf Weight Ratio
NAR	Net Assimilation Rate
NARC	National Agricultural Research Centre
NIAB	Nuclear Institute for Agriculture and Biology
NUFFIC	Netherlands Organization for International Cooperation in Higher Education
NWFP	North West Frontier Province
RGR	Relative Growth Rate
SLA	Specific Leaf Area
WAPDA	Water and Power Development Authority

CHAPTER 1



Chapter 1

General introduction

Global saline arable lands

In many parts of the world, a considerable area of irrigated arable land has been facing a serious problem of salinization. Estimates have shown that around 6% of the global land area suffers from salinization due to natural causes or irrigation, posing a major strain on agricultural production. It is estimated that irrigation related salinization leads to the abandonment of 10^7 hectares of agricultural land annually (Frans *et al*., 2001). Out of the total arable and potentially arable land, about 25 % lies in the arid and semi arid climatic zones (O'Toole and Chang, 1979). Szabolcz (1991) has reported that 10 % of the world's land surface is estimated to be affected by salinity and sodicity. In Africa, 43.6 million hectares of salt-affected land has been estimated to exist (Dudal and Purnell, 1986). The main cause of degradation of fertile land into unproductive saline land in the semi-arid and arid zones is low precipitation combined with high evapotranspiration, which leads to accumulation of salts at the soil surface by capillary action (Malcolm, 1989). In addition, seepage of water from canals resulted in a shallow water table causing both waterlogging and salinity. In Pakistan, out of the total 6.2 million hectares saline-sodic soil, the cultivated portion is 45% and the rest is lying barren (GOP, 2003a). The soils that have surfaces of patchy salinity/ sodicity, between 0.6 million ha and 1.23 million ha are moderately saline soils (Kahlown and Khan, 2002). This results in an annual loss in productivity of crops of more than US $ 150 million to the nation.

Salt-affected arable land in Pakistan

In Pakistan, out of 15.6 million ha of canal-irrigated land 6.8 million ha land is reported to be salt-affected to variable degrees (Ahmad and Chaudhry, 1988). The economic cost of reclamation of these soils is equal to US $ 25 ha^{-1} yr^{-1} (Sandhu and Qureshi, 1986; Qureshi and Mahmood, 1989). The extent of salt-affected soils varies between provinces of the country as reported by various agencies and summarized in Table 1. Saline soils contain soluble salts. Saline soils can be reclaimed by heavy irrigations, but the availability of irrigation water is a constraint. However, sodic and saline-sodic soils pose other, perhaps more severe problems during reclamation. Cultivation of salt tolerant plants is another option to economically utilize these salt-affected soils. Screening of cereal crops (wheat, rice, barley) for salt tolerance and their cultivation in the moderately saline soils has improved crop productivity (Nawaz, *et al*., 1986). The severely salt-affected soils could be cultivated with highly salt tolerant plants (halophytes). During the winter, there is shortage of fodder for cattle in Pakistan (Akram, 1986). According to one estimate there are 105 million heads of cattle in Pakistan (GOP, 2003). Their fodder requirements are met from various fodder crops and crop residues and the rest from forage grazing (38 %), post harvest grazing (3 %), oilcakes, meals, animal protein (2 %) and cereal by-products (6 %), (GOP, 2002). Fodders are grown on fertile soils that cover 11 % of the total cropped area (2.52 million ha) in the country. The total fodder production from this area is 56 million tons (GOP, 2002). Pakistan has 8.4 million ha of salt-affected land, out of which 6.8 million ha is severely salt-affected and proves uneconomic for cereal crops production (Muhammad, 1990).

In Pakistan sugarbeet is cultivated on non-saline soils. The farmers in the sugarbeet growing areas are compelled to feed their cattle with the cuttings of leaves of sugarbeet during this period. Due to the high sugar content in the leaves of sugarbeet, the health of cattle may be affected (Niazi and Rozema, 2003). Moreover, the cutting of sugarbeet leaves for cattle feeding also results in a net low sugar content in the beet. Unlike sugarbeet, fodderbeet is salt tolerant at the vegetative growth stage and can be successfully grown in the salt-affected areas. The leaves and beet of the fodderbeet contain a markedly lower sugar content compared to sugarbeet (Quin *et al.*, 1980) and the health of the cattle will not be affected. It will help the utilization of otherwise deserted areas where no other crop can be grown.

Halophyte plant growth in saline environments

Terrestrial plants that can grow under highly saline soil conditions are called halophytes. Halophytes are found in land and coastal saltmarshes e.g. *Spartina anglica* and *Elymus pycnanthus*. Adaptations of halophytes to salt-affected environments may include a resistance to high salt concentrations in the rooting medium. Resistance mechanism, however, comes at a cost and halophytes typically show a low biomass production compared to non-halophytes (Rozema and Van Diggelen, 1991). Some halophytes naturally occure across vast areas of salt-affected rangelands and they have been grazed or browsed by animals for a long time. Halophytic grasses, shrubs and trees are all potential sources of fodder. The use of halophytes for rehabilitation and reclamation of salt-affected lands has been proven to be feasible. About one quarter of the world halophytes are (Chenopodiaceae) (Aronson, 1989). The most salt-tolerant higher plants include succulent members of that family, such as *Salicornia, Suaeda and Atriplex* species (Rozema, 1995). Of the fodderbeet genus *Beta*, Seabeet (*Beta vulgaris* subsp. *maritima*) is a halophyte that also belongs to that family. Fodderbeet (*Beta vulgaris* subsp. *vulgaris*) cultivars are grown in coastal areas of the Netherlands, the United Kingdom, Germany and other parts of Europe (Rozema, 1991). Successful cultivation of fodderbeet in the saline desert patches of Saudi Arabia and other Middle East countries has been realized (Ahmad and Ismail, 1996). Fodderbeet (*Beta vulgaris* subsp *vulgaris* cultivars) is more sensitive to salinity at germination compared to the later growth stages. *Beta vulgaris* subsp. *maritima*, a coastal halophyte and the ancestor of sugarbeet, fodderbeet, red beet and white beet, is naturally grown in coastal belts of many European countries. Fodderbeet (*Beta vulgaris* subsp. *vulgaris*) is more salt tolerant than many other fodder crops. This may directly and indirectly support the production of food in saline arable areas. Being low in sugar content, fodderbeet has considerable nutritional value for cattle feeding in the areas where an alternative suitable staple crop is difficult to be grown.

Experimental research on fodderbeet

Research studies were carried out at The Vrije Universiteit (Amsterdam, The Netherlands) on the germination, growth and physiology of fodderbeet and seabeet. Seed germination is a primary process that plays an important role prior to vegetative growth of a plant. Suitable environmental conditions are required for seed germination. The seed of plants germinate well in moist, non-saline soils but in saline conditions germination of seeds is significantly hampered due to the presence of high concentrations of salts. Salinity and high temperatures are primary limiting environmental conditions that significantly restrict the successful cultivation of crops in irrigated arid and semi-arid regions (Bergmeyer and Brent, 1974). In addition, the variability of the temperature response to germination of fodderbeet seeds is insufficiently documented.

Growth and physiology of fodderbeet and seabeet

The growth of the most of the plant species is reduced under salt stress conditions that do not reduce growth of halophytes (Aslam, *et al*., 1986; Malcolm, 1989). Different morphological and physiological parameters of halophytes are considered as indices of adaptation to saline environments.

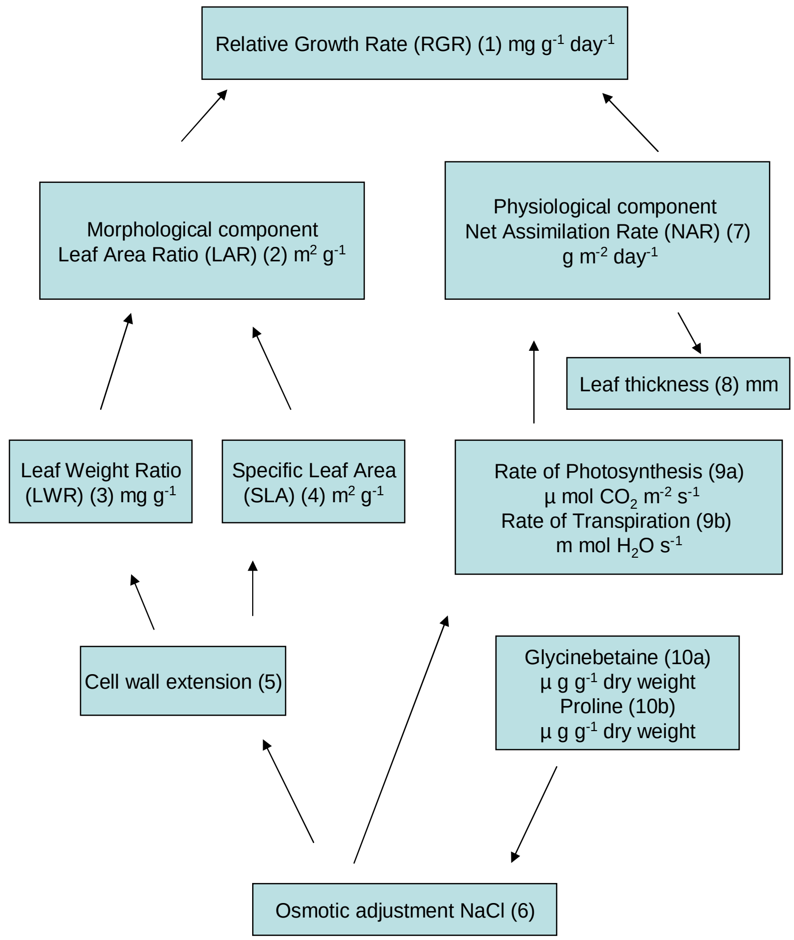
An increase in the relative growth rate (RGR) **(1)** of the plant is the product of two growth components, the leaf area ratio (LAR) **(2)** which is a morphological component of the plant growth and the net assimilation rate (NAR) **(7)** which is a physiological component (RGR = NAR * LAR). The morphological component further depends upon two sub components, leaf weight ratio (LWR) **(3)** and specific leaf area (SLA) **(4)**. These components affect the accumulation of photosynthetic material in the leaf. The adjustment of osmotic potential under increasing salinity, allowing sufficient water uptake may in the end result in a higher LWR **(3)** and SLA **(4)** due to the cell wall extension in the halophytes. Consequently the increase in the LWR and SLA increase the LAR **(2)** (LAR = LWR*SLA), which is a morphological component of plant growth. The increase in the NAR (7) of the plant under salt stress is related to the rate of photosynthesis **(9a)** and the rate of transpiration **(9b)**. A higher rate of transpiration relates to water transport to photosynthesising organs (leaf), which enhances the rate of photosynthesis. The difference between the rate of photosynthesis and the rate of respiration determines the net assimilation rate, the NAR (Lambers *et al*, 1989). The NAR is also indirectly related to increases in the leaf thickness. The thickness of the leaf is related to the extension in the cell wall due to a higher turgor pressure on the cell wall. An increase in the number of spongy parenchyma and palisade parenchyma layers may also result in increased leaf thickness. This may be associated with an increase in chlorophyll content of leaves when expressed per unit of leaf area. The increase in layers of photosynthetic cells in the leaf also enhances light interception, therefore, the photosynthate is increased. A higher concentration of salts in the root medium may disturb the activity of enzymes in the plant which results in the disturbance in the osmotic adjustment of cells under stress. In several plant species, a positive correlation between the leaf osmotic potential and the content of glycinebetaine, ß-alaninebetaine and prolinebetaine has been observed (Rhodes and Hanson, 1993; Grieve and Mass, 1984). Some proteins are also involved in the osmotic regulation of the plant plasma membranes (Babakov, *et al.*, 2000; Bunney, *et al.*, 2001; Bulychev, *et al.*, 2005). These organic compounds are known to have osmoprotective effects on the cells (Bartels and Sankar, 2005; Taji, *et al*., 2002; Maruyama, *et al*, 2004; Cram, 1976).

Plants may accumulate compatible osmolytes like glycinebetaine **(10a)**, proline **(10b)** or certain sugars under osmotic stress (Rabbani, *et al*., 2003). Eight times higher proline content under salt stress in *Petunias* has been reported compared to control plants by Bartels and Sanker (2005). The glycinebetaine is thought to protect the plant under stress by maintaining the water balance between the plant cell and its environment and by stabilizing macromolecules like proteins and sugars (Chen and Murata, 2002; Rontein, *et al*., 2002). The interrelationships of growth parameters are presented in Fig. 1 and are discussed in chapter 3 (solution-culture study at 0, 200 and 400 mM NaCl salinity), chapter 4 (i. Pot-culture study with four fodderbeet cultivars at 0 and 150 mM NaCl salinity, ii. Pot-culture study at 0 and 200 mM NaCl salinity with fodderbeet and seabeet, and iii. Pot-culture study at 0, 200 and 400 mM NaCl salinity with fodderbeet and seabeet). In chapter 5, the results of i) Field study in saline-sodic soil of Pakistan with four cultivars of fodderbeet, ii) Comparison of non-conventional fodderbeet biomass production with those of conventional fodder crops i.e. barley and oats, and iii) A pot-study on the growth of fodderbeet under saline-sodic soil, amended with farm yard manure, has been discussed.

Under saline-sodic conditions, due to excessive amounts of exchangeable Na^+, high Na^+/K^+ and Na^+/Ca^{2+} ratios are prevalent in the soil (Grieve and Fujiyama, 1987). Therefore, under salt stress conditions, plant take up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} is considerably reduced. Reasonable amounts of both K^+ and Ca^{2+} are required to maintain the integrity and functioning of cell membranes (Wexnue *et al*., 2003; Snedden and Fromm, 2001). The underlying mechanism for maintenance of adequate uptake of K^+ in plant tissue under stress seems to be dependent upon selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and distribution in the shoot (Munns *et al*., 2000; Carden *et al*., 2003).

The studies on fodderbeet cultivation in Pakistan were conducted at the Sugar Crops Research Station, Mardan, and the Arid Zone Research Sub-station, Ratta Kulachi, Dera Ismail Khan, N.W.F.P, Pakistan (Fig 2). A number of experiments were conducted on these sites. Results of two field experiments are described in the thesis. In one investigation, four fodderbeet cultivars (*Beta vulgaris* subsp. *vulgaris* cvs. Monored, Monoval, Majoral and Polygroeningia) were grown at the Sugar Crops Research Station, Mardan. The growth data on fresh weight of plant were recorded. The plant samples were analyzed for the chlorophyll content, sugar, protein and proline content. The fodder production of two conventional fodder crops (barley and oat) was compared with fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) grown in a saline-sodic field. The yield data including number of leaf, leaf area, fresh weight and chlorophyll content were collected from this experiment. Saline-sodic soil was collected from a research station located in Punjab province of Pakistan (Fig 2) and was transported to the National Agricultural Research Centre, (NARC) Islamabad, and non saline soil was collected from a field at NARC to conduct a pot experiment in the greenhouse to compare

Figure 1. Interrelationships of Fodderbeet (*Beta vulgaris* subsp. *vulgaris cultivars*) and Seabeet (*Beta vulgaris* subsp. *maritima*) plant growth parameters assessed in chapters 3, 4 and 5



the performance of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) grown in a saline-sodic soil amended with farm yard manure. Growth and ion relations were studied in this experiment. Results of these studies are presented in chapter 5 of this thesis.

Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and Seabeet (*Beta vulgaris* subsp. *maritima*) cultivation on salt-affected land in Pakistan

Fig 2. Map of Pakistan showing locations of experimental fields

Research goals and outline of the thesis

The plants grown under saline conditions experience a higher salt concentration (mainly sodium chloride) in the growth medium. Due to the higher salts concentrations in the soil solution, the root cells tend to experience an outward flow of water and the turgor pressure of the root cells is reduced, therefore, physiological drought with a low water potential is created in the plant. Halophytes overcome this situation by either accumulating of inorganic NaCl in their vacuoles by excluding it from their cells. Although the plants may tolerate the salinity by some mechanisms, the presence of high salt in the growth medium often affects the growth of the plant. Generally the survival, plant height, yield (Noble, *et al*., 1992), leaf area (Franco *et al*., 1993), leaf injury (Munns, 1993), relative growth rate (Cramer *et al*., 1990) and relative growth reduction (He and Cramer, 1992) are considered important parameters to study salt tolerance of plants. The relationship between some of these parameters is explained in Fig 1.

The seeds of glycophytes and halophytes show a reduction in germination rate under saline conditions (Shannon and Grieve, 1999). Miyamoto *et al*., (1985) reported a decline of 50 % germination in tomato and carrot seed at a root medium salinity of 12 and 18 dS m^{-1} respectively. Fodderbeet is reported to be salt sensitive at germination, however, it is salt tolerant at vegetative growth stage. The germination rate of seed of four fodderbeet cultivars (*Beta vulgaris* subsp. *vulgaris* cultivars Monored, Monoval, Majoral and Polygroeningia) were tested under different temperatures (20 ^{o}C, 25^{o}C and 30 ^{o}C) and at variable salinity (4, 8, 12, 16 and 20 dS m^{-1}). The results are presented in chapter 2 of this thesis.

Halophytes tolerate the saline conditions and show a resistance to higher salt concentrations with a reduction in growth rate. Different cultivars of the same plant had different behaviour toward salt tolerance (Flowers and Hajibagheri, 2001; Qadir *et al*., 2001). The reduction in growth of plants can be measured by observing the morphological and physiological growth parameters i.e. RGR, NAR, photosynthesis, leaf thickness of plant under higher concentration of salts. Biomass production of sugarbeet grown on salt-affected soils is significantly reduced; however fodderbeet is tolerant to saline conditions. The test plants can be subjected to different salinity levels in solution culture for their degree of salt tolerance. The salt concentration in the solution culture can be maintained easily. A solution culture experiment was performed to study the growth and ion response of fodderbeet and seabeet under increasing salinity (0, 200 and 400 mM NaCl). The data based on the growth response and ion relations obtained from this experimentation are discussed in chapter 3.

In a solution culture experiment, plants were provided with nutrients along with various salt concentrations. Depending on the composition, the nutrients in solution culture may positively or negatively affect plant growth under saline conditions. Salinity conditions in soils are different from those in solution culture, but the maintenance of salt concentrations and pH in soil is difficult compared to that in solution culture. While in solution cultures nutrients may become depleted nutrient availability in commercial garden soil used for pot-culture experiments, is usually higher than in nutrient solution. Therefore, the fodderbeet plant response to salinity was also studied in pot-culture under controlled conditions. Three greenhouse experiments were conducted in pot-culture to explore the growth response of fodderbeet and seabeet under increasing salinity. In the first experiment, three fodderbeet cultivars and seabeet were grown under control and 150 mM NaCl concentration in garden soil in the greenhouse. The biomass production, growth parameters and physiological components, e.g. net photosynthesis and leaf thickness, were recorded in this study. In a second experiment, a higher salinity level was increased to 200 mM NaCl to record the effect of high salinity on the growth and physiology of fodderbeet and seabeet. In addition to biomass production, the ion concentration and level of some osmolytes (sugars, protein, glycinebetaine) were also analyzed to assess their role in salt tolerance of fodderbeet and seabeet for a growth period of 5 weeks. In a third pot-culture experiment, fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) were subjected to 0, 200 and 400 mM NaCl salinity. Growth and water relations of fodderbeet and seabeet were studied in this experiment for two weeks of plant growth. The data of morphological and physiological growth parameters in response to salinity from the three greenhouse experiments is presented in chapter 4.

The growth response of fodderbeet was also studied under field conditions in Pakistan (chapter 5). Four cultivars of fodderbeet were grown in saline-sodic soil in Pakistan to screen for suitable cultivars for propagation in Pakistan. The data on biomass production and physiological components of growth, chlorophyll and osmolyte levels were recorded. A comparison of biomass production of a non-conventional fodder crop (fodderbeet) and two conventional fodder crops (barley and oat) was made in a field experiment under saline-sodic soil conditions in Pakistan. Different soil amendments are being used in salt-affected areas of Pakistan (Ahmad *et al.*, 1988). In a pot-culture study, farm yard manure was added as an amendment to saline-sodic soil and its effect on the growth of fodderbeet was assessed. Growth and physiological parameters were recorded in this experiment. The findings of these three field experiments on fodderbeet in Pakistan are discussed in chapter 5.

Table 1. Salt-affected and waterlogged area (thousand hectares) in Pakistan

	Directorate of Land Reclamation (1968-69)					**WAPDA**			**Soil Survey of Pakistan (1976-84)**	
	Area Surveyed	**Salt-affected**	**%**	**Waterlogged**		**Salt-affected**	**Waterlogged** 0-5 ft	**Waterlogged** 0-10 ft	**Salt-affected**	**Waterlogged**
Punjab	8830	1270	14	70		2440	610	3340	2120	1160
Sind	5530	860	15	140		5340	-	3240	2140	930
NWFP	400	40	10	10		130	50	120	620	50
Baluchistan	780	50	6	1		-	-	20	-	-
Pakistan	15540	2220	14	230		7910		6720	4880	2140
	Colombo Plan (1952-54)					Revelle *et al*. (1964), (Average, 1949-59)			Muhammad (1973)	
	Gross Area	**Salt-affected**	**%**	**Waterlogged**	**%**	**Cultivated area**	**Salt-affected**	**%**	**Salt-affected**	**Waterlogged**
Punjab	11490	2290	20	1790	16	9540	1180	12	2400	440
Sind	7450	4180	56	2800	38	4480	1460	33	1930	870
NWFP	570	30	5	-	-	-	-	-	510	80
Baluchistan	-	-	-	-	-	-	-	-	-	-
Pakistan	19510	6520	33	4590	24	14020	2640	19	4840	1390

(Muhammad, 1990)

CHAPTER 2

Chapter 2

The germination response of fodderbeet to salinity

Abstract

Seed germination of four fodderbeet cultivars (*Beta vulgaris* subsp.*vulgaris* cv. Monoval, Majoral, Monored and Polygroeningia) was studied using different salinity levels (4, 8, 12, 16, 20 dS m^{-1} NaCl) at 20^{o}, 25^{o} and 30 ^{o}C temperature conditions. The highest germination rate of fodderbeet seed was noted under non-saline conditions. Seed germination of fodderbeet decreased with increased salinity. At the highest salinity level applied, (20 dS m^{-1}), reflecting a salinity of about 40% of seawater (~200 mM NaCl) the fodderbeet seed germination rate was about 20%. The seed germination rate slightly varied with temperature (20 ^{o}C, 25 ^{o}C and 30 ^{o}C). Fodderbeet seed germination at 25 ^{o}C was somewhat higher than at 20 ^{o}C and 30 ^{o}C. Among all cultivars, germination performance of cv. Monoval at increased salinity and higher temperature (25 ^{o}C), was comparatively better than that of *Beta vulgaris* subsp. *vulgaris* cvs. Majoral, Monored and Polygroeningia. The results of fodderbeet seed germination are discussed in relation to sowing, germination and fodderbeet cultivation in saline arid areas of Pakistan.

Introduction

Germination of seeds in a saline environment represents a vulnerable stage in the reproductive cycle of flowering plants. A low water potential of a saline environment hinder water uptake and inhibit growth and development of an emerging seedling. Fodderbeet cultivation in saline-sodic flood plain soils of Pakistan implies seed germination and seedling establishment in saline, sometimes sodic soils. The electrical conductivity (EC_e) of salt-affected arable land in Pakistan may be up to 20 dS m^{-1} **(**~ 200 mM NaCl).

In this chapter the seed germination of fodderbeet was studied in response to salinity and temperature.

Salinity and temperature stresses are primary limiting environmental conditions that significantly restrict the successful cultivation of crops in irrigated arid and semi-arid regions (Bergmeyer and Brent, 1974). Salt-affected non-arable land is increasing in canal-irrigated areas in North West Frontier Province (N. W. F. P.), Pakistan due to the rise of the water table resulting from the seepage from canal beds. Currently about 12 million hectares land is under moderate to high salinity and sodicity (Muhammad 1990). Production of almost every conventional crop in this area is significantly reduced under saline soil conditions. Hence the introduction of non-conventional salt tolerant fodder crops could be a suitable option. Successful stand establishment is one of the most difficult challenges for sugarbeet growers. Saline soil conditions increase the difficulty further. Sugarbeet is among the salt tolerant crops, but it is reported to be less salt tolerant at germination and emergence (Kaffka *et al*., 2001). Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is known to be sensitive during germination but tolerant to increased salinity during later growth stages (Bernstein and Hayward, 1958). Also, fodderbeet has been reported to be salt tolerant at the vegetative growth stages (Niazi, *et al.* 1997). Fodderbeet is cultivated in the coastal areas of European countries as well as in New Zealand (Furunes, 1988; Goh and Magat, 1989). Application of NaCl as fertilizer, for improved growth and biomass of the crop have been reported by Draycott and Bugg (1982); Magat and Goh (1988).

The germination studies presented in this chapter were conducted to explore the response of four cultivars of fodderbeet to increased salinity and at different temperatures. These studies help to explore the conditions for germination of cultivars of fodderbeet and fodderbeet cultivation in saline arable land as well as in arid zones of Pakistan.

Materials and Methods

Four fodderbeet (*Beta vulgaris* subsp. *vulgaris*) cultivars Monored, Monoval, Majoral and Polygroeningia were received from Holland through the courtesy of Department of Ecology and Ecotoxicology, Free University, Amsterdam. *Beta vulgaris* subsp. *vulgaris* cultivars Monored, Monoval and Majoral have monogerm seed, while that of *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia is multigerm, consisting of clusters of seed. Initially the seed germination rate was tested in 0 mM NaCl. Later seeds of the cultivars were tested for germination rate under saline conditions. Salt solutions were prepared in ¼ strength Hoagland solution (Hoagland and Arnon, 1950). One hundred eighty pairs of petri dishes (Pyrex) were thoroughly washed with distilled water, rinsed with de-ionized water and dried in an oven. Three layers of filter paper were placed in each petri dish and then separated into four groups of 45 petri dishes each. These were divided into five sub-groups for the various salinity treatments. A number of 900 seeds of each of the four cultivars of fodderbeet were counted. Seeds of each cultivar were further divided into five groups, each group containing 60 seeds. Twenty seeds of each cultivar were placed in a petri dish on filter paper at almost equal distances from each other. Each plate was weighed on a top loading balance. NaCl salt solutions with EC 4, 8, 12, 16 and 20 dS m^{-1} (1 dS m^{-1} ~ 10 mM NaCl) were prepared in de-ionized water. An equal volume of salt solution was added to the dishes to maintain the concentration of salt treatment constant and the weight of each plate with the cover was recorded. Three groups of plates were placed in separate incubators which were maintained at three different temperatures (20^{o}, 25^{o} and 30° C ±1^{0} C). Water losses were compensated after every six hours with de-ionized water by maintaining the initial weight of petri-dishes including experimental material. Germination rates were recorded after one week. A seed was considered germinated when at least a 2 mm long radicle developed. Data obtained were subjected to analysis of variance test using SPSS version 10.0. One-way ANOVA was carried out per fodderbeet cultivar per factor according to Little and Hills (1978). A one-way ANOVA for the germination of seed of each fodderbeet cultivar and each temperature i.e. 20^o, 25^o and 30^o C was computed separately.

Results

Effect of salinity on seed germination

The seeds of all the fodderbeet cultivars germinated at the third day of the experiment except *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia, which germinated on the fourth day. The seed germination rate under 0 mM NaCl was 100 %. The percent seed germination was significantly reduced ($P<0.01$) in all the cultivars with increasing NaCl concentration (Table 1-3). The highest seed germination rate (75-94 %) of all cvs. was found at minimum salinity level (4 dS m^{-1}). At the highest salinity level i.e. 20 dS m^{-1}, germination varied from 17-39% for the four cultivars. In contrast with the *Beta vulgaris* subsp. *vulgaris* cv. Majoral, the lowest germination rate was recorded in *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia at 20 dS m^{-1} at all the temperatures. The germination behaviour of *Beta vulgaris* subsp. *vulgaris* cv. Majoral was more variable compared to other cultivars.

Effect of temperature on seed germination

Maximum percentage of all fodderbeet cultivars was observed at 25^o C (Table 2) varying from 88-94% at 4 dS m^{-1}. At 30^o C the percent germination varied from 76-81% at 4 dS m^{-1} (Table 3). Seed germination of fodderbeet cv. Majoral was reduced significantly beyond the EC level of 12 dS m^{-1} and statistically the same percentage was observed under temperature regimes of 20^o and 25 ^{o}C. However, at 30^o C the seed germination of the same cultivar was low at the highest level of salinity (20 dS m^{-1}). The other cultivars showed a uniform decrease in seed germination with increase of NaCl salt stress at all temperature regimes. The fodderbeet (*Beta vulgaris* subsp. *vulgaris* cultivars) Majoral and Monoval performed similarly at 25 ^{o}C (Table 2) while a similar trend was observed with cultivars Monored and Monoval under temperature of 20^o and 30 ^{o}C (Table 1 & 3).

Table 1. Effect of various salinity levels (dS m^{-1}) on the germination rate % of four cultivars of fodderbeet at 20 ^{o}C (3 replications of 20 seeds per petri dish). Means with different letter(s) in the same column differ significantly at P<0.01

Salinity	Fodderbeet cultivars			
EC dS m^{-1} (NaCl)	*Beta vulgaris* subsp. *vulgaris* cv. Monored	*Beta vulgaris* subsp. *vulgaris* cv. Majoral	*Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia	*Beta vulgaris* subsp. *vulgaris* cv. Monoval
4 (~ 40 mM)	82.3 a	75.3 a	82.0 a	82.7 a
8 (~80 mM)	51.0 b	56.0 b	59.7 b	58.0 b
12 (~120 mM)	41.3 c	38.0 c	40.3 c	43.0 c
16 (~160 mM)	38.7 c	38.6 bc	36.0 d	42.3 c
20 (~200 mM)	19.0 d	21.3 c	17.0 e	29.7 d
LSD	3.8	1.8	2.5	2.6

LSD = Least Significant Difference

Table 2. Effect of various salinity levels (dS m^{-1}) on the germination rate % of four cultivars of fodderbeet at 25 ^{o}C (3 replications of 20 seeds per petri dish). Means with different letter(s) in the same column differ significantly at P<0.01

Salinity	Fodderbeet cultivars			
EC dS m^{-1} (NaCl)	*Beta vulgaris* subsp. *vulgaris* cv. Monored	*Beta vulgaris* subsp. *vulgaris* cv. Majoral	*Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia	*Beta vulgaris* subsp. *vulgaris* cv. Monoval
4 (~ 40 mM)	94.0 a	90.9 a	90.7 a	87.7 a
8 (~80 mM)	60.0 b	57.0 b	58.7 b	53.7 b
12 (~120 mM)	32.8d	38.0 c	41.0 c	42.0 c
16 (~160 mM)	38.3 c	38.5 c	39.3 c	39.7 c
20 (~200 mM)	32.0 d	32.4 c	28.7 d	39.0 c
LSD	3.5	7.2	2.8	3.0

LSD = Least Significant Difference

Table 3. Effect of various salinity levels (dS m^{-1}) on the germination rate % of four cultivars of fodderbeet at 30 ^{o}C (3 replicates of 20 seeds per petri dish). Means with different letter(s) in the same column differ significantly at P<0.01

Salinity	Fodderbeet cultivars			
EC dS m^{-1} (NaCl)	*Beta vulgaris* subsp. *vulgaris* cv. Monored	*Beta vulgaris* subsp. *vulgaris* cv. Majoral	*Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia	*Beta vulgaris* subsp. *vulgaris* cv. Monoval
4 (~40 mM)	81.3 a	78.6 a	76.3 a	79.3 a
8 (~80 mM)	58.3 b	57.0 b	58.0 b	56.3 b
12 (~120 mM)	35.7 c	42.4 c	44.3 c	45.3 c
16 (~160 mM)	22.7 d	31.5 d	32.7 d	36.0 d
20 (~200 mM)	20.0 d	20.4 e	16.7 e	29.3 d
LSD	2.8	9.6	2.8	7.7

LSD = Least Significant Difference

Discussion

An inhibition of seed germination under saline conditions in plants has been previously reported by Waisel (1972). A low germination rate of seed can play a decisive role in the establishment of plants in a saline environment and consequently the biomass production may be negatively affected. Fodderbeet is a fodder crop grown in the coastal areas of European countries. Fodderbeet cultivars (*Beta vulgaris* subsp. *vulgaris*) originate from the seabeet (*Beta vulgaris* subsp. *maritima*), a coastal halophyte from the Atlantic European coast and from the coastline around the Mediterranean (Rozema, 1995). There is considerable variation in the response of seeds of plants to increased salinity, but generally seed germination decreases with increasing salinity. Tobe *et al*. (2000) reported that the seed germination rate of *Kilidium capsicum* (a halophyte; Chenopodiaceae) incubated with a -0.8 MPa NaCl solution was 73, 80 and 54 % at 10, 20 and 30 ^{o}C, respectively, but all radicles died before their length exceeded 5 mm. In contrast, when seeds were incubated with a -0.8 MPa PEG solution at 20 ^{o}C, 68 % of seeds germinated, and 95 % of the emerging radicals survived beyond 5 m.m. Seedlings are the most vulnerable stage in the life cycle of plants, and germination determines when and where seedling growth begins (Gutterman, 1993; Kigel, 1995; Ungar, 1995; 1996; Ramoliya and Pandey, 2002 & 2003). Therefore appropriate germination responses of halophytic species to environmental parameters determine there distribution in saline environments. Qureshi *et al.* (1980) reported 80 percent seed germination at 15 dS m^{-1} of wheat (*Hordeum vulgare*) grown in the plains of Punjab province of Pakistan. The percent germination in wheat varied with the wheat variety and the type of the salt used during the experiment. A decline of 50 percent germination in tomato and carrot seed at a root medium salinity of 12 and 18 dS m^{-1} respectively has been reported by Miyamoto *et al.* (1985). Reduced germination with increased salinity may relate to the toxicity of ions present in the saline medium (Uhvits, 1946) and to the decreased water availability to seeds (Chapman, 1968). Ryan *et al.* (1975) noted that chloride ions in solution along with Na^{+} ions restrict germination more than a similar concentration of Ca^{2+}. Increased salinity caused a low rate of seed germination due to high osmotic pressure in a study comparing germination of four grasses (Ryan *et al*., 1975). A slight improvement in alfalfa seed germination at low osmotic potential and under different temperatures (21^{o} to 33 ^{o}C) was reported by Stone *et al.* (1979). At the optimal temperature regime of 20-30 ^{o}C, *Sporobolus isoclades* seeds showed 93 % germination at 0 mM NaCl. The germination rate decreased to 18 % with increasing salt concentration to 500 mM NaCl (Gulzar and Khan, 2002). Temperature shifts may affect a number of processes determining germinability of seeds including membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994). Better germination ability of seed of *Arabidopsis* under saline or mannitol treatment, has been reported by Villalobos *et al*., (2004). A significant decline in the germination rate of *Beta vulgaris* subsp. *vulgaris* cv. Majoral seeds beyond 17 dS m^{-1} NaCl salinity at 25^{o} C has been reported under controlled conditions by Steen and Schoehuijs (1995). The seed germination of *Beta vulgaris* subsp. *vulgaris* cv. Majoral was higher than that of *Beta vulgaris* subsp. *vulgaris* cv. Polyproductiva. The seed germination of polygerm fodderbeet cultivars like *Beta vulgaris* subsp. *vulgaris* cv. Polyproductiva under saline conditions has been reported to be significantly lower compared to *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monoval. Germination of *Beta vulgaris* subsp. *vulgaris* cv. Majoral seeds at salinities higher than 200 mM NaCl have been reported by Huisman and Naus (1992).

Seed germination of the coastal halophyte *Salicornia* is highest at 0 mM NaCl, while adult plants grow well at seawater salinity (about 500 mM NaCl). Like in most glycophytes (Rozema, 1975), germination of many halophytes is highest at non-saline conditions and it is gradually reduced with increased salinity (Rozema, 1975) while the salt tolerance of the vegetative halophyte plant may be considerable. In coastal saline areas halophyte seed germination occurs during the wet season when rainfall temporarily reduces the salinity of soil moisture near the surface. Seed germination of fodderbeet may resemble that of its ancestor, seabeet (*Beta vulgaris* subsp. *maritima*), which naturally occurs along the drift line of the Atlantic and Mediterranean coasts. After germination during the wet winter half-year seabeet seedlings will have successfully established (Rozema, 1975). In the present study temperature was kept constant and no diurnal change occurred. The seed germination rate was high (80-90 %) and varied slightly with temperature (20 ^{o}C, 25 ^{o}C and 30 ^{o}C). Fodderbeet seed germination at 25 ^{o}C was somewhat higher than at 20 ^{o}C and 30 ^{o}C. The high germination rate of fodderbeet seeds at elevated temperatures may partly be an adaptation to the moderate (Atlantic coast) and warm climate (mediterranean coast of Europe and Africa).

At the salinity level of 20 dS m^{-1} (200 mM NaCl) roughly equalling 40% of the salinity of seawater (500 mM NaCl), the response of fodderbeet seed germination was about 20%. Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cultivars) seed germination of different cultivars was high (94 – 75 %) at low salinity level (4 dS m^{-1}) (Table 1- 3) at temperatures from 20 ^{o}C - 30 ^{o}C. Based on the seed germination response to salinity and temperature variation, fodderbeet could be grown as a winter crop by direct sowing in the field in Pakistan.

Soil salinity in the arable soils of Pakistan usually ranges from 5 to 20 dS m^{-1} (Qureshi *et al*., 1980). The germination rate of fodderbeet at 5 dS m^{-1} (50 mM NaCl) was ranged from 60 - 90 % and 30 - 40 % at 20 dS m^{-1} and 25 ^{o}C. It can therefore be expected that direct sowing of fodderbeet seed during the winter half year on saline arable land of Pakistan will lead to considerable germination. In addition, direct sowing of the multigerm seed of *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia may lead to more emerging seedlings in the saline arable land. However, this may also imply (time-consuming and cost of labour) thinning of the number of young fodderbeet plants.

During the monsoon (the rainy season), heavy rains wash away a considerable quantity of salt in the saline areas. Part of the salt dissolves in the rain water and leaches to the water table, thus soil salinity becomes low. Therefore, during the winter period the soil salinity is lower than during the rest of the year. Sowing of fodderbeet seed in the winter may therefore result in better germination. Temperatures ranging from 20 ^{o}C – 30 ^{o}C are prevailing in Pakistan during winter season. The seed germination studies presented in this chapter indicate that direct sowing of fodderbeet seed in the winter period may lead to considerable germination of fodderbeet seedlings in the salt-affected area of Punjab and upper Sind provinces.

CHAPTER 3

Chapter 3

Growth and physiological response of fodderbeet and seabeet to salinity

Abstract

A comparison was made between the growth of the coastal halophyte seabeet (*Beta vulgaris* subsp. *maritima* L.) and the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) in response to salinity. About 21 % reduction in relative growth rate of seabeet (*Beta vulgaris* subsp. *maritima*) and 29 % in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) was observed at NaCl salinities of 400 mM NaCl in the rooting medium as compared to non-saline medium. On a dry weight basis at 0 mM NaCl salinity, a 25 % higher relative growth rate in the case of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral, 200 mg g^{-1} day^{-1}) was obtained when compared with seabeet (*Beta vulgaris* subsp. *maritima*, 150 mg g^{-1} day^{-1}). Although, the Net Assimilation Rate (NAR) was not affected by increased salinity, both species showed 35 % reduction in Leaf Area Ratio (LAR). The decrease in the leaf area per unit of plant biomass is explained by a marked reduction of the Specific Leaf Area (SLA) of 47 % in for fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral), while leaf thickness increased significantly with increasing salinity. The Leaf Weight Ratio (LWR) was similar at all salinity levels. There was no significant effect of increased salinity on the rate of photosynthesis. Similarly, no marked differences between seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) were observed in the uptake of sodium, potassium, calcium and magnesium even at high salinity.

Introduction

Salt tolerance of plants varies greatly during different phases of growth and development. Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral), a species with relatively high salt tolerance during vegetative growth, is successfully grown as a fodder crop in the coastal areas of many European countries (Magat and Goh 1988; Rozema, 1991; 1995). The plant (top and beet) is used as a valuable source of fodder for cattle. Feeding of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) to dairy cows significantly improved fat and protein content of milk and the yield (Roberts, 1987). Fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral has been reported to be a salt tolerant plant up to 150 mM NaCl during the vegetative growth period (Niazi *et al.* 1995). The growth response of the plant appears to be characteristic of that of halophytes, because NaCl added to the soil aids its successful growth (Draycott and Bugg 1982). Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is reported to be rich in sucrose (Theurer *et al*., 1987) but dry matter yield is low when it is cultivated under saline conditions (Beringer *et al*., 1986). Yield of sugarbeet is markedly reduced in the presence of salinity in the growth medium. Therefore, sugarbeet production on salt-affected land is not economical. In contrast, fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) is tolerant to salinity during vegetative growth and yields higher dry matter under saline conditions (Niazi *et al*., 1995), but sucrose content is low (Quin, *et al*., 1980). Seabeet (*Beta vulgaris* spp. *maritima)* a biennial coastal halophyte, and ancestor of sugarbeet, fodderbeet, red beet and white beet, is naturally grown in coastal belts of European countries. A comparative study of *Beta vulgaris* spp. *maritima* and domesticated *Beta vulgaris* subsp. *vulgaris* cv. Majoral grown under saline soil conditions could give an idea about its successful cultivation in Pakistan as well. Hence, a study on these beets was planned under artificially salinized conditions to evaluate a potential fodder crop especially for small farmers of salt-affected lands in developing countries like Pakistan.

Materials and methods

Seed collection

Seeds of *Beta vulgaris* subsp. *maritima* were obtained from the coast of Brittany, France, near Plouger**n**eau, about 20 km north of Brest. The fruits were collected in September 1989. The fruits were removed from the flower stalk, cleaned, dried and stored in a cool dark room. Fruits of *Beta vulgaris* subsp. *vulgaris* cv. Majoral were kindly obtained from the seed company, Zwaan en de Wiljes, Scheemda , The Netherlands. Fruits of *Beta vulgaris* subsp. *vulgaris* cv. Majoral are genetically monogerm, and that of the natural coastal halophyte, *Beta vulgaris* subsp. *maritima* are polygerm, which implies that every clump of fruits contains about three seeds.

Pre-cultivation and experimental growth conditions

Fruits were germinated by placing in moist quartz sand, frequently moistened with demineralized water. Germination usually occurred within 4-5 days for the *Beta vulgaris* subsp. *vulgaris* cv. Majoral, while for seabeet (*Beta vulgaris* subsp. *maritima*) germination could be delayed and is more variable. Seedlings were established in a NaCl-free nutrient solution for one week. From week 2 the seedlings were grown on a solution of 50 mM NaCl which was replaced every two days. One seedling with a shoot height of 4 cm was transferred to each black plastic (polyethylene) pot filled with 2.7 liters of 0.5 strength aerated Hoagland’s solution that was refreshed two times per week. The plants were grown in a greenhouse, (25 ^{o}C day, 21 ^{o}C night temperature, 70 % relative humidity). Illumination was provided by HPI/T lamps (Philips) with about 250 µEinstein m^{-2} s^{-1} (PAR). Daylight was 12 hour. Salinity of the nutrient solution was varied at three levels, 0, 200 and 400 mM NaCl. Plants were analyzed and harvested at 20 and 30 days after the final concentration of 400 mM NaCl had been reached. Growth parameters were calculated by using an MS Excel Spread Sheet after Lenssen (1993).

Plant analysis

Leaf thickness was measured with a Dial pipe gauge (0.01-10 mm) No-2046-08-Mitutoyo-Japan. Net photosynthesis was measured on single leaves clipped in a Parkinson Leaf Chamber of a portable ADC-LCA3 system (The Analytical Development Company Ltd., Hoddesdon, Herts, UK) at a photon flux density of about 250 µEinstein m^{-2} s^{-1} (PAR). Leaf area was measured using a Li-3100 area meter (Li-Corp. Inc., Lincoln Nebraska, USA). The concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were determined by flame emission and atomic absorption spectrophotometry after acid digestion of dried plant material in a concentrated HNO_3 : $HClO_4$ (7:1, v/v) mixture.

Statistical analysis

Analysis of variance (ANOVA) was carried out according to procedures given in Sokal and Rohlf (1981).

Results

Analysis of growth

The mean relative growth rate of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) at 0 mM NaCl is 25 % higher than that of the seabeet (*Beta vulgaris* subsp. *maritima*) (Table 1). The mean relative growth rate of seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) was reduced by 21 and 29 %, respectively, with increasing salinity to 400 mM NaCl. However, the mean relative growth rate of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) at 200 mM NaCl was statistically non-significant under 0 and 200 mM NaCl.

Table 1. Relative Growth Rate and growth parameters of seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) cultivated in nutrient solution with different salinities (mM NaCl). Means of three replicates, with standard error mean. The growth parameters were calculated based on harvest at 20 and 30 days after the start of experiment. 'Change' indicates changes in the values obtained at 400 mM NaCl compared to the control plants (0 mM NaCl). One-way ANOVA for each parameter was computed separately for fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). Figures followed by different letters in the same column are significantly different for each parameter (P<0.05). Lettering is ranked in descending order.

Salinity mM NaCl	**RGR mg g^{-1} day^{-1}**	**NAR g m^{-2} day^{-1}**	**LAR m^2 g^{-1}**	**LWR g g^{-1}**	**SLA m^2 g^{-1}**
Seabeet (*Beta vulgaris* subsp. *maritima*)					
0	151.3 ±1.7 a	6.69 ±0.9 b	0.020 ±0.0018 a	0.843 ±0.014 a	0.024 ±0.002 a
200	140.5 ±7.2 a	10.45 ±1.3 a	0.018 ±0.0023 a	0.835 ±0.009 a	0.022 ±0.003 a
400	118.9 ±5.7 b	7.17 ±0.7 b	0.013 ±0.0009 b	0.838 ±0.003 a	0.015± 0.004 b
	-21%	-7 %	-35 %	-0.60 %	-36 %
Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral)					
0	200.9 ±18.1 a	7.08 ±0.9 b	0.027 ±0.0019 a	0.91 ±0.011 a	0.030 ±0.002 a
200	212.3	7.08	0.022	0.90	0.024

	±11.2	±1.0	±0.0005	±0.005	±0.001
	a	b	a	a	b
400	142.6	7.97	0.017	0.90	0.016
	±11.7	±0.7	±0.0004	±0.012	±0.003
	b	a	b	a	c
	-29 %	+12 %	-35 %	-0.5 %	-47 %

RGR = Relative Growth Rate, NAR = Net Assimilation Rate, LAR = Leaf Area Ratio, LWR = Leaf Weight Ratio, SLA = Specific Leaf Area.

The mean Net Assimilation Rate (NAR) was significantly increased with increased salinity (200 mM NaCl) in seabeet (*Beta vulgaris* subsp. *maritima*). There was a significant increase in NAR observed in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) under 400 mM NaCl level. At 0 mM NaCl the Leaf Area Ratio (LAR) as well as the Specific Leaf Area (SLA) of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) was 35 and 25% higher than the values measured for the seabeet (*Beta vulgaris* subsp. *maritima*). Accordingly the thickness of the leaves of seabeet at 0 mM NaCl is less than the leaf thickness of foddereet (*Beta vulgaris* subsp. *vulgaris* Majoral) (Table 2). The LAR was reduced by about 35% with increasing salinity for both subspecies. The SLA of seabeet (*Beta vulgaris* subsp. *maritima*) showed a decline of 36% with increased salinity and the reduction of SLA for fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was 47%. The Leaf Weight Ratio (LWR) was about 0.84 for seabeet (*Beta vulgaris* subsp. m*aritima*) and 0.90 for fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and salinity did not affect the LWR in either of the two subspecies. The leaves of seabeet (*Beta vulgaris* subsp. *maritima*) are 0.4 mm thick at 0 mM NaCl and with increasing salinity the thickness is raised to 0.56 mm. At 0 mM NaCl the leaves of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) are thinner (0.3 mm) than that of seabeet (*Beta vulgaris* subsp. *maritima*) (0.4 mm) while at 400 mM NaCl the thickness of

Table 2. Leaf thickness (μm) of leaves of seabeet *Beta vulgaris* subsp. *maritima* and fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral at different salinities (mM NaCl) of the nutrient solution measured 30 days after the start of the experiment. Average values of 48 replicates with standard error of the mean. One-way ANOVA was separately computed for seabeet *Beta vulgaris* subsp. *maritima* and fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Figures followed by different letters in the same row are significantly different for different salinity levels (P<0.05). Lettering is ranked in descending order.

	Salinity levels (mM NaCl)		
	0	**200**	**400**
Beta vulgaris subsp. *maritima*	408 ±18 c	446 ±23 b	559 ±21 a
Beta vulgaris subsp. *vulgaris* cv. Majoral	292 ±13 c	378 ±14 b	493 ±17 a

Table 3. Net rate of photosynthesis (μmol CO_2 m^{-2} s^{-1}) of leaves of *Beta vulgaris* subsp. *maritima* and of *Beta vulgaris* subsp. *vulgaris* cv. Majoral grown in nutrient solutions at diffenet salinities (mM NaCl) measured 20 days after the start of the experiment. Means and standard error of three replications. One-way ANOVA was separately computed for seabeet *Beta vulgaris* subsp. *maritima* and fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Figures followed by different letters in the same column are significantly different for different salinity levels (P<0.01). Lettering is ranked in descending order.

mM NaCl	***Beta vulgaris* subsp. *maritima***	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**
0	1.46 ±0.04 a	1.52 ±0.05 b
200	1.59 ±0.09 a	1.46 ±0.03 b
400	1.12 ±0.07 b	1.79 ±0.04 a

Table 4. Concentrations of Na^+ and K^+ (upper part) and Ca^{2+} and Mg^{2+} (µmol g^{-1} dry weight) in the shoots of seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) at different salinities (mM NaCl) of the nutrient solutions. Means and standard error of the mean of three replications. One-way ANOVA was separately computed for seabeet *Beta vulgaris* subsp. *maritima* and fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Figures followed by the different letters in the same row are significantly different for different salinity levels (P<0.01). Lettering is ranked in descending order.

Ions		**mM NaCl**		
		0	**200**	**400**
Na	*Beta vulgaris* subsp. *maritima*	22 ±0.3 b	473 ±2.7 a	559 ±2.4 a
	Beta vulgaris subsp. *vulgaris* cv. Majoral	46 ±0.9 b	481 ±2.9 a	609 ±2.6 a
K	*Beta vulgaris* subsp. *maritima*	318 ±2.1 a	96 ±0.9 b	87 ±0.8 b
	Beta vulgaris subsp. *vulgaris* cv. Majoral	227 ±1.5 a	96 ±0.7 b	89 ±0.7 b
Ca	*Beta vulgaris* subsp. *maritima*	39 ±0.7 a	12 ±0.03 b	13 ±0.04 b
	Beta vulgaris subsp. *vulgaris* cv. Majoral	26 ±0.4 a	6 ±0.02 b	6 ±0.02 b
Mg	*Beta vulgaris* subsp. *maritima*	38 ±0.6 a	16 ±0.03 b	12 ±0.03 b
	Beta vulgaris subsp. *vulgaris* cv. Majoral	32 ±0.4 a	20 ±0.02 b	14 ±0.03 c

the fodderbeet leaves had increased to 0.50 mm. Hence the difference of the leaf thickness between the fodderbeet and seabeet was reduced i.e. 0.50 and 0.56 mm respectively.

However, the rate of net photosynthesis expressed as µmol CO_2 m^{-2} s^{-1} was not significantly affected by increased salinity up to 200 mM NaCl in either of the two subspecies (Table 3) but a significant increase was observed due to higher salinity (400 mM NaCl) level in *Beta vulgaris* subsp. *vulgaris* cv. Majoral, while a significant decrease observed in *Beta vulgaris* subsp. *maritima* at 400 mM NaCl.

The concentrations of sodium in the leaves increased in both the sub-species to about 500 - 600 µmol g^{-1} on dry weight basis with increasing salinity (Table 4). The concentrations of potassium of the leaves of both the sub-species showed a decline from 350 - 250 µmol g^{-1} on dry weight basis at 0 mM NaCl to about 85 µmol g^{-1} on dry weight basis at 400 mM NaCl.

The concentrations of calcium and magnesium of the leaves was reduced with increasing salinity of the culture solution (Table 4).

Discussion

Salt tolerance of Beta vulgaris

Beta vulgaris subsp. *maritima* is a halophyte, occurring at the upper part of salt marshes along the Atlantic and Mediterranean coast line. It is the ancestor of sugarbeet, fodderbeet, red beet and white beet. The highly productive cultivars of the beet are the result of breeding programs that have selected for high growth rate, a biennial life cycle, extended development of the tap root, sugar content, nutritional value for cattle and human consumption during the past decades (Roberts, 1987).

In the present study it has been demonstrated, that the salt tolerance assessed as the ratio of the mean relative growth rate at 400 mM NaCl and at 0 mM NaCl of the halophyte coastal seabeet *Beta vulgaris* subsp. *maritima* is about 10% higher than in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). In the analysis of growth parameters it appears that the Leaf Area Ratio and Specific Leaf Area are most affected with increasing salinity in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) (Table 5). Under saline conditions, the growth of halophytes is generally stimulated and accompanied by concentrations of Na and Cl ions particularly in the leaves (Flowers *et al*., 1977; Watkins *et al*., 1988). Accumulation of these ions in the leaves may be an adaptation, which provides this plant with considerable flexibility to survive fluctuating NaCl concentrations in its environment. The concentration of K^{+}, Ca^{2+} and Mg^{2+} were decreased by salinization. Several features of the growth response to NaCl may also be adaptations to saline conditions. Watkins *et al*. (1988) have also reported similar results.

At 0 mM NaCl, the characteristics of the relatively thin leaves of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) seem to be associated with the high SLA, while for the seabeet the thicker leaves relate to a smaller SLA. This outcome indicates that breeding for highly productive beet cultivars has involved primarily changes of the morphological growth parameters LAR and SLA (Lambers *et al*., 1998) rather than the physiological growth parameter NAR. On the other hand, the relatively high salt tolerance of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) is associated with only a small decline in the mean relative growth rate compared to the coastal

Table 5. Comparison of characteristics of seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) grown in nutrient solution and garden soil with increasing salinity (0, 200, 400 mM NaCl). The data is taken from two different experiments or comparison of the growth parameters. The original data discussed elsewhere (Niazi, *et al*., 2000; Niazi and Rozema, 2003)

Characteristics	**Seabeet (*Beta vulgaris* subsp. *maritima*)**	**Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral)**
Poly/monogerm	Polygerm	Monogerm
Number of leaves plant^{-1}	20 – 50	8 – 13
Leaf thickness (µm)	400 – 500	300 – 500
Salt tolerance	High	Slightly reduced
Relative growth rate (mg g^{-1} day^{-1})	150 – 120	200 – 140
Net assimilation rate (g m^{-2} day^{-1})	No change with salinity	No change with salinity
Leaf area ratio (m^2 g^{-1})	0.020	0.027
	35 % reduction with salinity	35 % reduction with salinity
Specific leaf area (m^2 g^{-1})	36 % reduction with salinity	47 % reduction with salinity
Leaf weight ratio (g g^{-1})	No change with salinity	No change with salinity
Net rate photosynthesis	No change with salinity	No change with salinity
Transpiration rate	No change with salinity	
	No difference between subspecies	
Na^+ and K^+ uptake	No difference between subspecies	

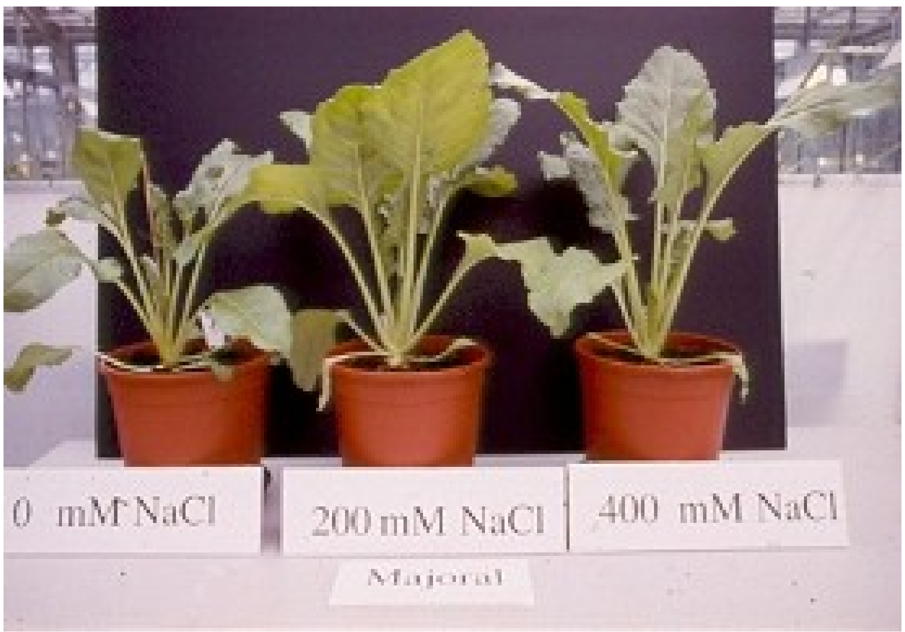
ancestor *Beta vulgaris* subsp. *maritima*, which is quite remarkable. Although RGR of fodderbeet is relatively more affected by salinity compared to seabeet, the growth rate of fodderbeet at the highest salt concentration is still higher than that of seabeet. It implies that breeding for productive beet cultivars has slightly reduced salt tolerance of this important crop plant. The increased RGR of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) at 200 mM NaCl in the nutrient solution was not significant (Rozema, 1990). Similar results have also been discussed by Niazi *et al*. (2005), Ashraf *et al*. (2003), Niazi *et al*. (2000) and Vivanco *et al*. (2002).

Extrapolation of our greenhouse experiments with nutrient solution to outdoor cultivation of fodderbeet in saline soil is difficult. However, field experiments with four cultivars of fodderbeet in Pakistan (Niazi *et al*., 2002; 2004) demonstrated increased growth of all cultivars tested in salt-affected arable land compared to non-saline control plots. In salt-affected arable land soil salinity does not exceed 70 mM (Qureshi *et al*., 1980). At this level of salinity, NaCl may stimulate growth of *Beta vulgaris* subsp. *vulgaris* cv. *Majoral* in comparison to non saline soil conditions (Wyn-Jones and Storey, 1981). As far as salinity is concerned fodderbeet seems to be a crop that can be successfully cultivated in salinized arable land where soil salinity will only rarely reach values of 200 mM NaCl. In addition to salinity, sodicity generally reduces the quality of arable land all over the world and in Pakistan in particular. It is not known how the salt tolerant crop *Beta vulgaris* subsp. *vulgaris* cv. *Majoral* will survive under anaerobic, impenetrable sodic soils lacking structure.

The basic mechanism of salt tolerance of *Beta vulgaris* has not been analyzed in this study, but presumably it will be similar to the mechanism of salt tolerance of the many halophytic members of the Chenopodiaceae (Ashraf and Harris, 2004). This comparative ecophysiological study offers the perspective that programs for domestication of natural inland and coastal halophytes are promising. Such breeding programs may lead to increased rates of growth of plants, while not eliminating salt tolerance. In search of possibilities of cultivation of (crop) plants in (highly) saline land, such as in salt-affected irrigated areas and in areas where only saline water is available, domestication of halophytes seems to be a hopeful line of agricultural and ecophysiological research.

In further reports (Niazi *et al*. 1997; 1998; 1999) more detailed analyses have been made of the salt tolerance of the seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral).

CHAPTER 4



Chapter 4

Growth analysis of fodderbeet in response to salinity grown in pot-culture under controlled conditions

Abstract

Three greenhouse experiments were conducted in pots using garden soil to explore the response of cultivars of fodderbeet to salinity. In experiment I three cultivars of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cvs. Majoral, Monored and Polygroeningia) and seabeet (*Beta vulgaris* subsp. *maritima*) were germinated in sand moistened with distilled water and transplanted in pots filled with garden soil with two levels of salinity (0 and 150 mM NaCl). Plants were harvested after 2 and 6 weeks of salinity treatment. Growth and some physiological parameters were recorded at the time of each harvest. Significant differences in Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) were noted with high salinity, but leaf thickness did not significantly change with salinity. Leaf area per plant in *Beta vulgaris* subsp. *vulgaris* cv. Majoral was significantly higher than in the other cultivars tested and the seabeet *Beta vulgaris* subsp. *maritima*. Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) were found to be better adapted to 150 mM NaCl concentration soil solution than the other cultivars. To test the behaviour of fodderbeet and seabeet plants under a higher salt concentration, a second experiment was conducted using garden soil without and with a higher salinity level (200 mM NaCl). Growth and ionic relations of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) were studied. Both species tolerated increased salinity. Various growth (number of leaves, leaf area) and physiological (rate of photosynthesis, transpiration, osmotic potential, water potential) parameters

were analyzed. In addition, ionic relations as well as the sugar and chlorophyll concentrations in plant parts of seabeet (*Beta vulgaris* subsp. *maritima*) and fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) were compared. The adaptation of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) to 200 mM NaCl indicates that fodderbeet cultivation on salt-affected arable land of Pakistan (soil salinity up to 20 dS m^{-1} ~ 200 mM NaCl will be feasible. Further, a third pot experiment was conducted under greenhouse conditions with fodderbeet and seabeet, subjecting the plants to salinity levels of 200 mM and 400 mM NaCl. The experiment was conducted for a two weeks period of plant growth. Various growth and physiological parameters (dry weight, leaf area, water relations and net photosynthesis) were recorded. The fodderbeet and seabeet responded differently in terms of growth parameters. Plant growth of both fodderbeet and seabeet was significantly reduced at 400 mM NaCl, while no significant growth reduction occurred at 200 mM NaCl as compared to 0 mM NaCl.

Introduction

Salinity is a limiting factor to crop production. The yield of most crops is decreased when cultivated in salt-affected areas. Generally soil salinity-sodicity problems are handled by chemical and biological methods. Chemical methods are used to reclaim sodic soils (Ahmad *et al*., 1985), while cultivation of salt tolerant species on salt-affected lands forms the basis of the biological reclamation. Identification of a wide variety of species with higher salt tolerance is important to achieve more success from this approach. The potential of saline land for the growth of salt tolerant plants has been documented (Malcolm, 1989). Kallar grass (*Leptochloa fusca*) and *Atriplex* spp. can be successfully grown on otherwise unproductive lands (Aslam *et al*., 1991). In Pakistan, the animal feed deficit during winter (November to February) ranges from 25 to 40 percent

(Akram, 1986). Therefore non-conventional salt tolerant fodder plants may be assessed to make up the fodder requirement of cattle and the objective of utilization of salt-affected soil be achieved as well (Rozema, 1991). Fodderbeet is a cultivar domesticated from seabeet that is found along the coastal areas of European countries under natural saline conditions. Fodderbeet can be introduced in Pakistan as a non-conventional fodder crop grown on saline soils. Fodderbeet cultivation may reduce the acute shortage of fodder for animals during winter. Fodderbeet is reported to be a salt tolerant crop at the vegetative stage (Rozema *et al*., 1992). It is cultivated in the coastal areas in many European countries such as the U. K., the Netherlands, France, Germany, Spain, Austria, and Sweden (Magat and Goh, 1988). A few reports on successful cultivation of fodderbeet are available from Russia and New Zealand (Popovic and Stikie, 1986; Magat and Goh, 1988). Application of NaCl (295-1180 kg ha^{-1}) is required for a significant increase in the tops and the root yield of fodderbeet (Goh and Magat, 1989). Although the halophilous nature of fodderbeet has been reported in literature (Magat and Goh, 1988), there is a little information about the role of various ions in the growth of fodderbeet. Cell wall extensibility is significantly affected by deposition of Ca^{2+} in the cell wall, either by forming cross bridges or by inhibiting wall-loosening enzymes (Cleland, 1986). The concentration of Ca^{2+} and Mg^{2+} in reclaimed soils was reported to be higher near the soil surface (Ahmed *et al*., 1985). Magnesium forms an important component of chlorophyll (Marschner, 1986) and uptake of magnesium in plants can indirectly improve the growth of plant by more chlorophyll synthesis and increased rate of photosynthesis.

Sugar yield of the sugarbeet was also reported to improve by the application of NaCl (Stephen *et al*., 1980; Pescini and McCrone, 1980; Hamid and Talibudeen, 1976). The sugar concentrations of fodderbeet are low compared to sugarbeet (Quin *et al*., 1980), which favours raising fodderbeet as a fodder crop under saline conditions. The type of soil plays an important role in the response to sodium chloride application in relation to sugar yield of sugarbeet (Beringer *et al*., 1986). Synthesis of protein and nucleotides were inhibited by increased salinity ultimately resulting in decreased plant mass due to inhibition in cell division and cell enlargement (Hartt, 1970). The inward K^+ channels in the barley root cells are linked with G-protein modulators (Wegner and de Boer, 1997; de Boer, 2002; Bunney, *et al*, 2001, Bunney, *et al*, 2002; Van den Wijngaard, *et al*, 2005). A comparatively higher concentration of chlorophyll in fodderbeet has been reported by Niazi *et al*. (1999); Maas, *et al*., (1986); Maas and Nieman, (1978) under saline soil conditions. Different plant species have shown salt sensitivity at various growth stages (Boardman, 1977; Niazi, *et al*., 1999 & 1999a). Chenopodiaceae and Gramineae (Poaceae) show different behaviour for biosynthesis of glycinebetaine, which is a quaternary ammonium compound accumulating in the leaves of a wide range of species (Boardman, 1977; Haeder and Mengel, 1972; Papp, *et al*., 1983). Accumulation of glycinebetaine increases considerably under saline environments, especially in species belonging to Chenopodiaceae or Gramineae (Poaceae) (Haeder and Mengel, 1972; Papp, *et al*., 1983). Glycinebetaine concentrations increased with increasing salinity (Rozema *et al*., 1982). The increase in glycinebetaine has been correlated with the salt stressed leaf expansion. Glycinebetaine is thought to protect the plant by maintaining the water balance between the plant cell and its environment and by stabilizing macromolecules (Chen and Murata, 2002; Rontein *et al*., 2002; Ashraf and Harris, 2004). Glycinebetaine functions as an effective compatible osmolyte in plants subjected to salt stress (Mansour, 2000).

Research aims

The present chapter presents the results of three greenhouse pot-culture experiments on the performance of fodderbeet and seabeet grown under saline soil conditions to understand the mechanism of salt tolerance. In the first pot-culture experiment three cultivars of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cvs. Majoral, Monored and Polygroeningia) and seabeet (*Beta vulgaris* subsp. *maritima*) were grown under control (0 mM NaCl) and high salinity (150 mM NaCl) in garden soil under controlled conditions. Morphological parameters (biomass production), growth parameters (leaf thickness) and physiological parameters (net photosynthesis) were assessed in this experiment. In the second pot-culture experiment, the salinity level in the growth medium was increased to 200 mM NaCl to test the growth of fodderbeet and seabeet.

The K^+ level in plant tissue interacts with some proteins and regulates the functions of some molecules to correct assembling of targeting ion channels (Sinnige, *et al*., 2005). Therefore, in this study measurement of ion concentrations (Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+}) and levels of some osmolytes (sugars, glycinebetaine and protein) of plant parts (root and leaf) was included in addition to biomass production of fodderbeet and seabeet. The growth of fodderbeet and seabeet was studied for 2 weeks in this experiment. In the third pot-culture experiment, fodderbeet and seabeet were subjected to three increasing levels of salinity (0, 200 and 400 mM NaCl) for a two weeks growth period. The growth and water relations of fodderbeet and seabeet were studied in this experiment. The results of these studies may help understanding the salt tolerance level and inter-relationships of morphological and physiological components of growth of fodderbeet and seabeet. Fodderbeet cultivation may help to solve the problem of fodder shortage in winter season for cattle in salt-affected areas in Pakistan (Abdullah, 1987). Cultivation practice of fodderbeet in some areas would also be helpful for the introduction of other salt tolerant crops during the summer season.

Materials and methods

Experiment I

Seeds of three fodderbeet (*Beta vulgaris* subsp. *vulgaris*) cultivars Majoral, Monored and Polygroeningia and of seabeet (*Beta vulgaris* subsp. *maritima*) collected from the Atlantic Coast of Brittany (see chapter 3, page 41) were germinated in sand moistened with distilled water. The emerged seedlings were counted for record of percent germination. After two weeks of germination, one seedling was transplanted to each pot containing 3 kg garden soil (characteristics given in Table 1). Sixteen replicates were maintained per treatment. Two salt levels (i.e. 0 and 150 mM NaCl) were applied after one week of establishment of the seedlings in pots. The salt solution was added on alternate days with increments of 50 mM NaCl, twice on the top of the soil and third time to the dish placed at the bottom of pot in order to obtain a uniform salt concentration throughout the soil profile. The plants were raised in the greenhouse with an average temperature of 24° C during the day and 22° C during the night. The relative humidity was maintained at 75 percent and the light intensity 250 $\mu E\ m^{-2}\ s^{-1}$ was provided by HPI/T lamps (Philips). An initial harvest was taken before the addition of salt. Two harvests were taken after two and six weeks of the application of the salt treatment, respectively. The experiment was designed according to a complete randomized block design. At each harvest, the parameters assessed were: dry matter yield, leaf area using a leaf area meter (Li 3100 Li-Crop. Inc. Lincoln, Nebraska USA), as well as measurement of length and width

Table 1. Physico-chemical analysis of the garden soil used in the pot-culture experiment (source: Jongkind Potgrond mengsel, Aalsmeer)

Physical Analysis	
Moisture Percentage	64.0
Percent Organic matter	70.0
Volume weight perlite granule	207.0
Pore volume	88.3
Volume % water by PF 1.5	54.8
Volume % air by PF 1.5	33.5
Chemical Analysis	
Organic matter	72.0
$CaCO_3$	2.5
pH H_2O	6.2
EC dS m^{-1}	1.4
Anion (mmol l^{-1} extract)	
Cl^-	0.7
SO_4^{2-}	3.2
PO_4^{3-}	0.8
NO_3^-	2.2
Cation (mmol l^{-1} extract)	
NH_4^-	3.1
K^+	2.9
Ca^{2+}	1.8
Mg^{2+}	0.8
Total Nitrogen %	5.3

of the leaf blade manually, leaf thickness (Dial pipe gauge, 0.01-10 mm, no 2046-08-Mitutoyo Japan), total water potential of the shoot was assessed with a Pressure Bomb, osmotic potential of sap expressed from the leaves was estimated with a vapour pressure osmometer 5100, Wescor Inc., USA and net photosynthesis with a Parkinson leaf chamber ADC-LCA3 system, The Analytical Development Company Ltd, Hoddesdon, Herts, UK, recorded at the time of each harvest. Correlation of leaf area was calculated by assessment with a leaf area meter and as well as measurement of length and width. Relative growth rate, LAR, NAR, SLA and SLW were calculated according to Hunt (1982). Results were statistically analyzed using ANOVA described by Sokal and Rohlf (1981). The results recorded during the first experiment showed that the fodderbeet cultivars tested did not show a significant decline in growth at 150 mM NaCl concentration in the growth medium. Therefore, a higher level of salinity (200 mM NaCl equal to 40 % of sea water) was chosen to explore the optimum level of salinity tolerated by fodderbeet and seabeet in a second pot cultivation experiment.

Plant growth and harvest

Seeds of two species of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral and *Beta vulgaris* subsp. *maritima*) were germinated in sand under greenhouse conditions as described in the first experiment. Ten-day-old seedlings were transplanted to pots kept in a greenhouse (one seedling per pot) filled with one kg garden soil (Table 1). Two salinity levels (0 mM NaCl (control), and 200 mM NaCl) were maintained in the pots. Salinity was increased stepwise by addition of 50 mM NaCl every two days one week after establishment of seedling as described earlier. There were 20 replications per salinity level. A first harvest was taken after seven days of maintaining the required salinity level in the pots and the subsequent harvests were taken with an interval of seven days between each harvest. Growth parameters were calculated using MS Excel Spread Sheet after Lenssen (1993).

Chemical analysis

Plant material was dried at 60 ^{o}C in an oven to a constant weight and was analyzed for cations (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) using an Atomic Absorption Spectrophotometer (AAS). Chloride was analyzed on chlor-o-counter (Marius Instrumenten, Utrecht, The Netherlands). Fresh plant material was analyzed for chlorophyll according to Knudson *et al*., (1977). Plant material (50 mg) was crushed with quartz sand using 2.5 ml of 80 % acetone. The sample was left in the dark (ice cold) for 3 minutes and then centrifuged for 10 minutes at 4000 rpm and the volume was made to 5 ml. The samples were also analyzed for chlorophyll 'a' and 'b'. The photochemical efficiency of photosystem II was calculated according to Oquist and Wass (1988). Dried plant material (100 mg) was heated with 10 ml de-ionized water for 2 hours at 90^{o} C in a water bath and filtered. The extract was then subjected to analysis of total sugars (Bergmeyer, 1978), proteins (Peterson, 1984) and glycinebetaine (Storey and Wyn-Jones, 1977).

The plant faces variable salinity levels during its growth in the salt-affected field conditions. The plant growth under pot culture experiences fluctuations in salinity levels during irrigation. The salinity levels decrease at the time of irrigation due to dilution factor, while the salt concentrations increase slowly with the evapotranspiration of plant and soil surface during growth. The concentration of salts in the salt-affected soils gradually increases after irrigation due to evapotranspiration in the arid regions. Therefore, plants grown in saline soils tolerate even higher salinity than at the time of sowing. With this in mind a pot experiment was conducted under controlled conditions in the greenhouse (temperature 30º / 25º C day/night, relative humidity 70%, light 250 µE m^{-2} s^{-1} supplied by Philips Holland HPI/T Lamp) to assess the growth of fodderbeet and seabeet at salinities of 200 and 400 mM NaCl. *Beta vulgaris* subsp. *vulgaris* cv. Majoral and *Beta vulgaris* subsp. *maritima* were selected as test plants on the basis of previous studies (Niazi *et al*. 1995). Seeds were germinated in plastic trays containing sand using quarter strength Hoagland solution. Ten-day-old seedlings were transplanted into pots (one seedling per pot) containing 1 kg of garden soil (Table 1). Three salinity levels 0 (control), 200 and 400 mM NaCl were applied with 20 replicates. The salt treatments were applied as described in the previous experiments. The pH of solutions was checked and maintained at 5.5 on alternate days. The solutions were replaced after first harvest.

An initial harvest was taken before application of salinity. Plants were sequentially harvested two times at one-week interval. Plant material was oven-dried at 80º C until a constant weight of the sample was obtained. The growth parameters were recorded at the time of each harvest and growth relations were computed according to Hunt (1982). For water relations the water potential was measured by pressure bomb and osmotic potential by vapour pressure osmometer (5100 Wescor Inc., USA). The rate of photosynthesis was recorded using the ADC-LCA 3 system (The analytical Development Company Ltd., Hoddesdon, Herts, UK) for each harvest.

Statistical analysis

The data were analyzed statistically using one-way ANOVA testing salt treatment per cultivar per harvest methods for the first and second experiments, while two-way analysis of variance was computed for the third experiment as described by Sokal and Rohlf (1981). Means were compared with the LSD multiple mean comparison test. Significance levels were computed at $P < 0.05$ unless stated.

Results

Experiment I

The seeds of all the fodderbeet cultivars were approximately 100 percent germinated under control (0 mM NaCl) conditions within seven days of sowing. *Beta vulgaris* subsp. *maritima* showed a delayed germination of about four days. The leaves of seedlings of *Beta vulgaris* subsp. *maritima* were comparatively dark green in colour. Mean relative growth rate (RGR, mg g^{-1} day^{-1}) of *Beta vulgaris* subsp. *vulgaris* cultivars Majoral, Monored and Polygroeningia shoot was higher than that of *Beta vulgaris* subsp. *maritima* at the first harvest (Table 2). At a later stage all fodderbeet cultivars showed a stunted growth and reduced size of stem. The leaves were directly emerging from the stems. Whereas, *Beta vulgaris* subsp. *maritima* produced well developed and branched stems, that were comparatively longer (maximum length 4 cm). The RGR did not show any significant difference for the salinity treatment up to the second week of salt added to the pots, but there were significant differences between the cultivars for RGR at harvest II. The *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia showed the highest RGR while *Beta vulgaris* subsp. *maritima* gave the lowest value (Table 2). Presence of NaCl (150 mM) in the growth medium had no effect on RGR up to two weeks. After 6 week, RGR was significantly lower compared to first harvest (Table 2). At harvest II, the presence of 150 mM NaCl had a significant increasing effect on RGR in all the cultivars compared to the control. However, in *Beta vulgaris* subsp. *maritima*, there was a significant decrease in RGR in the presence of 150 mM NaCl compared to the control. Net assimilation rate (NAR, g m^{-2} day^{-1}) in *Beta vulgaris* subsp. *maritima* and *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia was higher than in *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monored (Table 2). NAR was increased by the salinity treatments for almost all the cultivars except *Beta vulgaris* subsp. *maritima* by the presence of 150 mM NaCl up to six weeks.

The leaf area ratio (LAR cm^{-2} g^{-1}) was not significantly different for the salinity treatment in all beet species up to 2 weeks. The LAR value of *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and that of Monored was higher than

Table 2. Effect of high salinity (150 mM NaCl in soil moisture) on growth parameters of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) after two (I) and six (II) weeks of treatments. Average values of 8 replications with standard error of the means. One-way ANOVA was computed per cultivar per harvest. Figures with different letters in the same column for each parameter differ significantly at P<0.05. Lettering is ranked in descending order.

mM NaCl	**Harvest**	**Fodderbeet cultivars**			**Seabeet**
		***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroe-ningia**	***Beta vulgaris* subsp. *maritima***
Relative Growth Rate (mg g^{-1} day^{-1})					
0	I	100.3 ±11.3a	97.2 ±11.9a	137.1 ±3.4a	72.5 ±3.4a
150	I	97.1 ±7.5a	95.31 ±9.1a	121.8 ±7.2a	80.2 ±3.4a
0	II	38.5 ±3.3b	28.4 ±1.1b	14.4 ±1.3b	50.0 ±5.1a
150	II	54.8 ±1.6a	44.5 ±4.9a	25.7 ±2.7a	39.6 ±3.9b
Net Assimilation Rate (g m^{-2} day^{-1})					
0	I	3.9 ±0.4a	3.78 ±0.7a	6.4 ±0.6a	6.78 ±1.2a
150	I	4.6 ±0.8a	4.5 ±0.6a	4.9 ±0.8a	7.02 ±0.4a
0	II	37.2 ±3.0b	20.5 ±1.1b	15.9 ±1.7a	52.8 ±2.3a
150	II	50.6 ±5.1a	45.7 ±3.4a	21.6 ±4.7a	47.9 ±1.4a
Leaf Area Ratio (cm^2 g^{-1})					
0	I	2.5 ± 0.3a	2.7 ± 0.4a	1.6 ± 0.1a	1.5 ± 0.2a
150	I	2.1 ± 0.1a	1.9 ± 0.1a	1.9 ± 0.2a	1.6 ± 0.1a
0	II	83 ± 7.0a	95 ± 11a	62 ± 7.0a	133 ± 20a
150	II	84 ± 1.0a	68 ± 14a	75 ± 9.0a	94 ± 11a
Leaf Weight Ratio (mg g^{-1})					
0	I	73.9 ± 3.9.0a	75.4 ± 1.5a	66.9 ± 4.3a	76.4 ± 1.9a
150	I	73.5 ± 4.8a	78.2 ± 2.7a	70.4 ± 3.7a	77.2 ± 1.2a
0	II	47.0 ± 1.0a	43.0 ± 6.0a	38.0 ± 8.0a	74.0 ± 1.1a
150	II	60.0 ± 1.4a	45.0 ± 1.3a	39.0 ± 4.0a	63.0 ± 2.0a
Specific Leaf Area (cm^2 g^{-1})					
0	I	35 ± 0.6a	35 ± 0.5a	23 ±0.1a	20 ± 0.3a
150	I	33 ± 0.3a	24 ± 0.1a	25 ± 0.2a	20 ± 0.1a
0	II	185 ± 15a	219 ± 27a	163 ± 7a	176 ± 14a
150	II	146 ± 27b	150 ± 12b	194 ± 5a	149 ± 13a
Water Potential (-Bars)					
0	I	9.2 ± 2.1a	6.7 ± 1.04a	11.4 ± 1.8a	13.7 ± 2.7b
150	I	11.5 ± 3.1a	9.7 ± 1.60a	10.2 ± 1.6a	19.5 ± 2.0a
Osmotic Potential (-Bars)					
0	I	9.7 ± 0.4b	10.4 ± 3.0a	8.3 ± 2.4b	17.6 ± 3.7b
150	I	14.4 ± 1.9a	12.9 ± 1.7a	12.6 ± 2.5a	23.1 ± 3.1a

that of *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia. A comparatively lower LAR in *Beta vulgaris* subsp. *maritima* (harvest II) had been recorded at 150 mM NaCl. The leaf area ratio increased at the time of second harvest compared to the first harvest (Table 2). The leaf weight ratio (LWR mg g^{-1}) was almost equal in all cultivars even with increased salinity (150 mM NaCl) up to 2 weeks. A significant positive correlation (R^2 = 0.96) was recorded in measuring leaf area by leaf area meter and by the product of leaf length and breadth. An increase in LAR after the week 6 may be associated with a decrease in LWR in all the cultivars except in *Beta vulgaris* subsp. *maritima*. A significant decrease in the specific leaf area (SLA, cm^{-2} g^{-1}) was noted in the presence of salinity in *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monored during harvest II (Table 2). The specific leaf area of *Beta vulgaris* subsp. *vulgaris* cv. Majoral and *Beta vulgaris* subsp. *vulgaris* cv. Monored significantly increased with the age of the plant (six weeks) but there were no significant differences among the cultivars and treatments in the initial two weeks period (Table 2). A similar trend was observed in the rate of net photosynthesis (μmol CO_2 m^{-2} s^{-1}) in *Beta vulgaris* subsp. *maritima,* which showed a significant higher rate of net photosynthesis in the high salinity treatment (150 mM NaCl) (Table 3). The total water potential of the shoot did not significantly change in the presence of salinity (150 mM NaCl) in all the cultivars except *Beta vulgaris* subsp. *maritima* where it increased significantly with increased salinity. NaCl did not affect the leaf thickness in the cultivars except in *Beta vulgaris* subsp. *maritima* (Table 3).

A marked reduction in the dry weight of root and leaf was noticed due to increased salinity after two weeks in all cultivars except for *Beta vulgaris*

Table 3. Effect of high salinity (150 mM NaCl in soil moisture) on net photosynthesis (μmol CO_2 m^{-2} s^{-1}) and leaf thickness (μm) of three

cultivars of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) after two weeks of salinity treatments. Average values of 8 replications with standard error of the means. One-way ANOVA was computed per cultivar per harvest. Figures with different letters in the same column for each parameter differ significantly at P<0.05. Lettering is ranked in descending order.

	mM NaCl	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia**	***Beta vulgaris* subsp. *maritima***
Net Photosynthesis	0	12.0±1.3a	14.1±1.0a	11.4 ±1.1a	12.9±1.4b
	150	13.9±1.2a	14.4±1.1a	10.2 ±1.2a	15.1 ±1.0a
Leaf Thickness µm	0	290 ±1a	280 ±10a	320 ±10a	410 ±10b
	150	280 ±10a	290 ±20a	300 ±10a	450 ±20a

Table 4. Percent increase (+) or decrease (-) in the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars and *Beta vulgaris* subsp. *maritima* cultivated at high salinity (150 mM NaCl) compared to the control (0 mM NaCl) in soil moisture after two (I) and six (II) weeks of the salinity treatments (Averages of 8 replications).

Cultivar	**Har-vest**	**Fresh weight (g)**			**Dry weight (g)**			**Leaf area (m^2)**
		Leaf	**Root**	**Total plant**	**Leaf**	**Root**	**Total plant**	
Beta vulgaris subsp. *vulgaris* cv. Majoral	I	- 14.7	-11.8	- 14.0	- 23.3	- 3.4	- 18.2	- 3.4
	II	+ 23.3	- 26.4	+ 4.1	+ 56.1	- 1.3	+ 24.4	+14.5
Beta vulgaris subsp. *vulgaris* cv. Monored	I	- 18.5	- 23.9	- 26.0	- 11.4	- 23.8	- 14.6	- 19.7
	II	+ 40.1	+ 30.2	+ 35.7	+62.2	+ 1.2	+ 26.6	+ 8.2
Beta vulgaris subsp. *vulgaris* cv. Polygroeningia	I	- 21.8	- 25.2	- 22.5	- 35.6	- 50.9	- 40.7	- 15.1
	II	+ 7.8	- 17.7	- 3.5	+ 9.2	- 13.4	- 11.8	+ 7.1
Beta vulgaris subsp. *maritima*	I	+ 22.0	+ 8.1	+ 19.3	+ 57.3	+ 50.6	+ 55.7	+13.8
	II	+ 1.9	+ 56.3	+ 11.6	+ 1.7	+ 49.5	+ 12.6	- 1.8

Table 5. Percent increase (+) or decrease (-) in the number of leaves of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cultivars) and seabeet (*Beta vulgaris* subsp. *maritima)* in the presence of 150 mM NaCl compared to the control (0 mM NaCl) after two (harvest I) and six (harvest II) weeks after the salt treatments. Percentages were calculated from the differences of values (increase or decrease in the number of leaf under 0 and 150 mM NaCl). (Averages of 8 replications).

Cultivar	**Harvest I**			**Harvest II**		
	0 mM	**150 mM**	**% ±**	**0 mM**	**150 mM**	**% ±**
Beta vulgaris subsp. *vulgaris* cv. Majoral	12.1	11.6	- 4.5	13.8	15.8	+ 14.5
Beta vulgaris subsp. *vulgaris* cv. Monored	12.9	12.3	- 4.8	14.0	12.3	- 12.5
Beta vulgaris subsp. *vulgaris* cv. Polygroeningia	10.3	11.9	+ 15.8	11.8	12.3	+ 4.3
Beta vulgaris subsp. *maritima*	21.9	23.3	+ 6.5	37.8	42.3	+ 11.9

subsp. *maritima* (Table 4). Leaf dry weight in *Beta vulgaris* subsp. *vulgaris* cv. Majoral showed an increase of 5 percent over the control treatment after 4 weeks of first harvest, there was a 1.3 percent reduction in root dry weight. *Beta vulgaris* subsp. *maritima* gained 57 percent leaf dry weight and 50 percent root dry weight over control after two weeks of treatment. A 55 percent increase of whole plant dry weight compared to the control treatment was recorded (Table 4).

Leaf area was reduced after two weeks of salt treatment in all the cultivars except *Beta vulgaris* subsp. *maritima* (Table 4). The number of leaves increased in the *Beta vulgaris* subsp. *vulgaris* cv. Majoral and in seabeet after six weeks (Table 5) while the leaf area per plant decreased (Table 4)

Experiment II

Statistically similar fresh biomass was produced by fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima)* up to 14 days while a significant increase in biomass of both the sub-species was observed due to increased salinity when harvested after 21 days. The dry matter accumulation of the shoots increased with the age of the plant i.e. there were no significant differences up to 28 days (Table 6), but the presence of salt enhanced the dry matter yield only after 35 days.

The fresh weight of the roots did not increase significantly ($P<0.01$) during the first 21 days. After that it increased significantly. Salinity treatment had no effect on fresh weight. The plants accumulated biomass during the first week of growth, showing an increase in the fresh weight of the roots in 200 mM NaCl compared to the control (0 mM NaCl). There was no significant increase in the dry weight of roots of fodderbeet and seabeet until the third week of plant growth. The plant tolerated the saline environments on fifth week of growth which is revealed by a significant decrease in the dry weight of roots under 200 mM NaCl compared to 0 mM (Table 7).

The shoot fresh /dry weight ratio was lower in saline conditions in *Beta vulgaris* subsp. *vulgaris* cv. Majoral but it increased with time. The shoot fresh/dry weight ratio in *Beta vulgaris* subsp. *maritima* was lower than that of fodderbeet in all the harvests taken. The ratio did not change in the later harvests. The root fresh/dry weight ratio of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) was significantly higher under the elevated salinity than that of the control, at day 21 only.

Table 6. Effect of high salinity (200 mM NaCl) at 5 subsequent harvests (days) on the fresh and dry weight of shoots (g) of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly differ between salinity treatments for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of fresh weight shoots, LSD salinity treatments = 6.67, Significance of dry weight shoots, LSD salinity treatments = 0.64, ($P<0.05$). Lettering is ranked in descending order.

Shoots fresh weight

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	2.7a	13.9a	21.7b	25.3b	27.0b
	200	2.7a	11.3a	30.6a	42.3a	44.8a
Beta vulgaris subsp. *maritima*	0	0.7a	4.8a	6.6b	10.7b	13.5b
	200	0.5a	2.7a	11.3a	25.1a	27.3a

Shoots dry weight

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.11a	0.57a	1.31a	3.21a	3.05b
	200	0.12a	0.54a	1.52a	3.09a	4.44a
Beta vulgaris subsp. *maritima*	0	0.04a	0.26a	0.53a	1.01b	1.64b
	200	0.03a	0.17a	0.63a	2.47a	2.48a

Table 7. Effect of high salinity (200 mM NaCl) at 5 subsequent harvests (days) on fresh and dry weight of roots (g) of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly differ between salinity treatments for the same cultivar and the the same harvest. (Averages calculated of 4 replications). Significance of fresh weight roots, LSD Salinity treatments = 5.43 Significance of dry weight roots, LSD Salinity treatments = 0.52, (P<0.05). Lettering is ranked in descending order.

Roots fresh weight

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.4a	3.0a	7.3a	17.1a	21.6b
	200	0.5a	2.3a	6.9a	16.2a	37.1a
Beta vulgaris subsp. *maritima*	0	0.2a	0.9a	2.4a	4.7a	12.5a
	200	0.2a	0.8a	1.8a	6.5a	10.2a

Roots dry weight

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.03a	0.21a	0.87a	2.49a	4.06a
	200	0.04a	0.16a	0.57a	2.42a	4.90b
Beta vulgaris subsp. *maritima*	0	0.02a	0.08a	0.36a	0.83a	2.49a
	200	0.02a	0.08a	0.21a	0.91a	1.95b

Table 8. Effect of high salinity (200 mM NaCl) on shoots fresh/dry weight ratios and roots fresh/dry weight ratios of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). The ratios were calculated from the averages of 4 replications. One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters in the same column and for each harvest differ significantly at P<0.05. Lettering is ranked in descending order.

		Shoots fresh/dry weight ratios		**Roots fresh/dry weight ratios**	
Harvest (days)	**Treatment mM NaCl**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *maritima***	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *maritima***
7	0	24.2a	17.2a	13.2a	9.2a
	200	22.0b	16.6a	12.2a	9.4a
14	0	24.7a	18.6a	14.4a	10.6a
	200	21.1b	16.0a	14.6a	10.5a
21	0	16.5b	12.5b	8.3b	6.7b
	200	20.1a	17.0a	12.1a	8.4a
28	0	11.0b	10.6b	7.2a	9.7a
	200	13.6a	13.3a	6.7a	7.1a
35	0	8.8a	8.2a	5.3a	5.0a
	200	10.1a	7.1a	5.4a	5.2a

Table 9. Sodium concentrations (µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at high salinity (200 mM NaCl) at 5 subsequent harvests (days) after salinity treatments. One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of sodium concentrations shoots, LSD Salinity treatments = 0.63. Significance of sodium concentrations roots, LSD Salinity treatments = 0.49, (P<0.05). Lettering is ranked in descending order.

Sodium concentrations shoots

Cultivars	mM NaCl	Harvest (days)				
		7	14	21	28	35
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	8.7b	9.0b	9.8b	9.9b	11.1b
	200	19.7a	24.9a	26.8a	25.9a	28.5a
Beta vulgaris subsp. *maritima*	0	9.5b	9.9b	10.7b	11.1b	11.5b
	200	18.0a	18.7a	19.5a	21.1a	22.5a

Sodium concentrations roots

Cultivars	mM NaCl	Harvest (days)				
		7	14	21	28	35
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	2.8b	3.0b	3.4b	3.5b	4.4b
	200	13.1a	13.4a	13.9a	14.5a	15.4a
Beta vulgaris subsp. *maritima*	0	3.3b	3.5b	3.8b	4.0b	3.9b
	200	9.9a	12.3a	14.9a	17.5a	24.1a

Table 10. Chloride concentrations (µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at 0 and 200 mM NaCl salinity at 5 subsequent harvests (days) after salinity treatments. One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of chloride concentrations shoots, LSD Salinity treatments = 0.73. Significance of chloride concentrations roots, LSD Salinity treatments = 2.42, ($P<0.05$). Lettering is ranked in descending order.

Chloride concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	5.6b	1.7b	2.7b	2.7b	3.0b
	200	35.8a	43.3a	44.4a	52.3a	67.4a
Beta vulgaris subsp. *maritima*	0	2.0b	2.2b	2.5b	2.8b	3.1b
	200	21.5a	23.2a	23.7a	27.0a	43.9a

Chloride concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	2.5b	3.2b	3.5b	3.6b	4.1b
	200	27.2a	28.5a	33.2a	36.0a	42.1a
Beta vulgaris subsp. *maritima*	0	1.5b	2.6b	2.5b	4.1b	4.3b
	200	66.1a	70.4a	85.8a	96.7a	140.8a

Ion concentrations

The concentration of Na^+ in fodderbeet and seabeet significantly ($P< 0.01$) increased in the presence of 200 mM NaCl in the root medium. The concentration of Na^+ increased with time in control plants (0 mM NaCl) as well. In general, the shoots had higher Na^+ concentrations than the roots (Table 9).

The Na^+ concentrations in the roots had increased with the age of the plants irrespective of treatment. *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoots contained higher Na^+ concentrations than *Beta vulgaris* subsp. *maritima.* Generally, a higher Na^+ concentration was recorded for plants at 200 mM NaCl compared to the control.

The effect of high NaCl concentrations in the growth medium resulted in significantly ($P< 0.01$) higher Cl^- concentrations in seabeet. Seabeet roots had a higher Cl^- concentration than the shoots (Table 10). Chloride concentrations in the roots and shoots increased with the age of the plant.

The potassium concentrations of the roots were generally lower in the presence of high saline conditions. The shoots contained significantly ($P<0.01$) higher K^+ concentrations than the roots (Table 11). Potassium ion concentrations decreased with age both in the shoots and roots. The concentrations of K^+ in the roots and shoots in the presence of salt were lower in seabeet than in *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Plant growth showed a significant negative correlation ($r^2 = -0.72$) with K ion concentrations in the plants.

Table 11. Potassium concentrations (µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of potassium concentrations shoots, LSD Salinity treatments = 0.26. Significance of potassium contcentrations roots, LSD Salinity treatments = 0.14, ($P<0.05$). Lettering is ranked in descending order.

Potassium concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	1.4a	1.4a	1.0a	0.8a	0.7a
	200	1.3a	1.2a	0.6b	0.7a	0.6a
Beta vulgaris subsp. *maritima*	0	1.5a	1.3a	1.1a	1.1a	0.9a
	200	1.3a	1.3a	1.2a	1.1a	1.0a

Potassium concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	1.0a	0.8a	0.8a	0.6a	0.5a
	200	0.6b	0.5b	0.4b	0.4b	0.3b
Beta vulgaris subsp. *maritima*	0	1.0a	1.0a	0.7a	0.6a	0.5a
	200	0.5b	0.5b	0.4b	0.4b	0.3b

Table 12. Calcium concentrations (µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of calcium and magnesium concentrations shoots, LSD Salinity treatments = 0.03. Significance of calcium and magnesium concentrations roots, LSD Salinity treatments = 0.32, ($P<0.05$). Lettering is ranked in descending order.

Calcium concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.03a	0.04a	0.05a	0.06b	0.08b
	200	0.05a	0.06a	0.06a	0.09a	0.10a
Beta vulgaris subsp. *maritima*	0	0.05a	0.07a	0.08a	0.08a	0.09a
	200	0.07a	0.08a	0.08a	0.09a	0.10a

Calcium concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.11a	0.17a	0.22a	0.31a	0.30a
	200	0.28a	0.29a	0.28a	0.23a	0.21a
Beta vulgaris subsp. *maritima*	0	0.27a	0.20a	0.19a	0.18a	0.17a
	200	0.35a	0.35a	0.28a	0.20a	0.20a

Table 13. Magnesium concentrations (µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities in the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of magnesium concentrations shoots, LSD Salinity treatments = 0.14. Significance of magnesium concentrations roots, LSD Salinity treatments = 0.12, ($P<0.05$). Lettering is ranked in descending order.

Magnesium concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	2.00a	2.01a	2.04a	2.02a	2.05a
	200	1.41b	1.48b	2.10a	2.11a	2.13a
Beta vulgaris subsp. *maritima*	0	1.86b	1.93a	1.94b	2.00b	2.01b
	200	2.03a	2.09a	2.15a	2.67a	2.70a

Magnesium concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.97a	0.94a	0.94a	0.87a	0.87a
	200	0.91a	0.87a	0.86a	0.82a	0.80a
Beta vulgaris subsp. *maritima*	0	1.10b	1.38a	1.17b	1.24b	1.26b
	200	1.37a	1.44a	1.49a	1.54a	1.70a

Table 14. Chlorophyll ‘a’ and ’b’ concentrations ((µg g^{-1} dry weight) in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of chlorophyll ‘a’ concentrations, LSD Salinity treatments = 0.015. Significance of chlorophyll ‘b’ concentrations, LSD Salinity treatments = 0.004, ($P < 0.05$). Lettering is ranked in descending order.

Chlorophyll ‘a’ concentrations

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.9a	0.9a	0.9a	0.9a	1.1a
	200	0.7a	0.9a	1.0a	1.0a	1.2a
Beta vulgaris subsp. *maritima*	0	0.7a	0.8a	0.8a	0.8b	1.0b
	200	0.7a	0.9a	0.9a	1.0a	1.3a

Chlorophyll ‘b’ concentrations

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.2a	0.3a	0.3a	0.3a	0.4a
	200	0.2a	0.3a	0.3a	0.3a	0.3a
Beta vulgaris subsp. *maritima*	0	0.2a	0.3a	0.3a	0.5a	0.5a
	200	0.2a	0.2b	0.2b	0.2b	0.3b

The concentrations of calcium in the roots were higher in the presence of salt (200 mM NaCl) compared to the control during the early growth period (Table 12). Calcium concentrations were significantly ($P < 0.01$) higher in seabeet roots than in *Beta vulgaris* subsp. *vulgaris* cv. Majoral roots during the first week of growth. Calcium concentrations significantly increased with the age of the plant in *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoots. Significantly higher Ca^{2+} concentrations in the shoots of seabeet were noted under saline conditions, at the age of 28 - 35 days.

The magnesium concentrations in *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoots were lower for the first two weeks under saline conditions. However, Mg^{+2} concentrations significantly increased in *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoots during 28-35 days in the presence of 200 mM NaCl (Table 13). The magnesium concentrations in fodderbeet shoots significantly ($P < 0.01$) increased after 21-35 days in the presence of 200 mM NaCl.

There were no significant differences recorded in the chlorophyll ‘a’ and ‘b’ concentrations of fodderbeet with high salinities (0 and 200 mM NaCl) during the whole growth period. There was a significant increase in the chlorophyll ‘a’ and chlorophyll ‘b’ concentrations observed in *Beta vulgaris* subsp. *maritima* after week 4of plant growth (Table 14) but chlorophyll ‘b’ decreased with increasing salinity even after second week (Table 14).

High salinity tended to decrease sugar concentrations in the roots in both fodderbeet and seabeet. The sugar concentrations in fodderbeet roots only increased at the salinity level of 200 mM NaCl during 28-35 days, while sugar concentrations in seabeet roots and shoots increased at 200 mM NaCl since the early growth period. The seabeet roots and shoots accumulated higher sugar concentrations than fodderbeet. The sugar concentrations in

Table 15. Sugar concentrations ((µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) with 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of sugar concentrations shoots, LSD Salinity treatments = 31.80. Significance of sugar concentrations in roots, LSD Salinity treatments = 472.9, ($P < 0.05$). Lettering is ranked in descending order.

Sugar concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	34.3a	46.0a	511.3a	869.0a	718.3a
	200	23.9a	28.1a	51.4b	182.0b	567.5b
Beta vulgaris subsp. *maritima*	0	39.7b	241.7a	447.0a	859.7a	1406.3a
	200	180.7a	256.9a	312.5b	346.4b	520.4b

Sugar concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	34.0a	76.3a	1209.7a	1506.7a	5547.0a
	200	42.9a	45.8a	75.2b	834.0b	1821.7b
Beta vulgaris subsp. *maritima*	0	118.0a	647.7a	1817.7a	1593.3b	2776.0a
	200	518.4a	866.9a	1868.0a	2292.7a	2517.7a

Table 16. Protein concentrations ((µg g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) with 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of protein concentrations in shoots, LSD Salinity treatments = 29.48. Significance of protein concentrations in roots, LSD Salinity treatments = 18.24, ($P < 0.05$). Lettering is ranked in descending order.

Protein concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	242.5b	244.7b	307.7b	234.6b	273.4b
	200	502.6a	457.4a	375.2a	294.2a	148.1a
Beta vulgaris subsp. *maritima*	0	233.3b	243.3b	519.8b	617.6b	656.5b
	200	524.1a	716.4a	770.6a	868.7a	960.8a

Protein concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	136.6b	146.4a	152.9b	76.8b	95.4b
	200	165.8a	90.8b	211.2a	283.0a	378.4a
Beta vulgaris subsp. *maritima*	0	168.4b	198.8b	246.8b	398.7b	475.2b
	200	297.4a	404.3a	483.5a	489.3a	586.8a

Table 17. Glycinebetaine concentrations (µmol g^{-1} dry weight) in shoots and roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) with 0 and 200 mM NaCl salinity at 5 subsequent harvests (days). One-way ANOVA for each cultivar and each harvest was computed separately. Figures with different letters significantly varied between salinities for the same cultivar and the same harvest. (Averages calculated of 4 replications). Significance of glycinebetaine concentrations shoots, LSD Salinity treatments = 0.45. Significance of glycinebetaine concentrations roots, LSD Salinity treatments = 0.98, ($P < 0.05$). Lettering is ranked in descending order.

Glycinebetaine concentrations shoots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	2.9b	2.6b	2.1b	1.8b	1.5b
	200	3.5a	4.8a	5.5a	6.3a	8.4a
Beta vulgaris subsp. *maritima*	0	6.2a	6.9a	7.0b	7.5b	8.0b
	200	6.1a	7.0a	14.2a	15.9a	16.5a

Glycinebetaine concentrations roots

Cultivars	**mM NaCl**	**Harvest (days)**				
		7	**14**	**21**	**28**	**35**
Beta vulgaris subsp. *vulgaris* cv. Majoral	0	0.2b	2.2b	2.8b	3.0b	3.7b
	200	3.5a	5.0a	6.5a	6.5a	7.9a
Beta vulgaris subsp. *maritima*	0	3.3a	5.0b	5.2b	5.9b	6.3b
	200	3.5a	7.8a	16.1a	19.3a	20.5a

fodderbeet and seabeet shoots were significantly lower upon salt treatment (Table 15). The sugar concentrations increased significantly in shoots of both cultivars during 21-35 days.

The protein concentrations increased with the age in fodderbeet root at salinity level of 200 mM NaCl. A significant increase in the protein concentrations of fodderbeet shoots was recorded at 200 mM NaCl. The protein concentrations of shoots were higher than that of the roots (Table 16). The protein concentrations of fodderbeet shoots decreased under high salinity with plant age after 14 days. On the other hand, the protein concentrations in seabeet increased with plant age. The protein concentrations in both roots and shoots of seabeet increased at salinity level of 200 mM NaCl. High protein concentrations were observed in seabeet compared to fodderbeet.

The glycinebetaine concentrations increased at 200 mM NaCl (Table 17). The glycinebetaine concentrations in seabeet and shoots were higher as compared to fodderbeet. A significant increase in glycinebetaine concentrations with increasing salinity was observed in seven-days-old fodderbeet plants (root and shoot), while glycinebetaine concentrations of the seabeet plants (root and shoot) increased significantly after 14 days.

Experiment III

Plant dry weight in *Beta vulgaris* subsp. *vulgaris* cv. Majoral did not change in response to high salinities (200 and 400 mM NaCl) during the first week but after two weeks the dry weight decreased with the increasing salinity (200 and 400 mM NaCl). The seabeet (*Beta vulgaris* subsp. *maritima*) produced comparatively lower dry weight compared to that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). The dry weight of seabeet (*Beta vulgaris* subsp. *maritima*) also decreased with the increase in the salinity

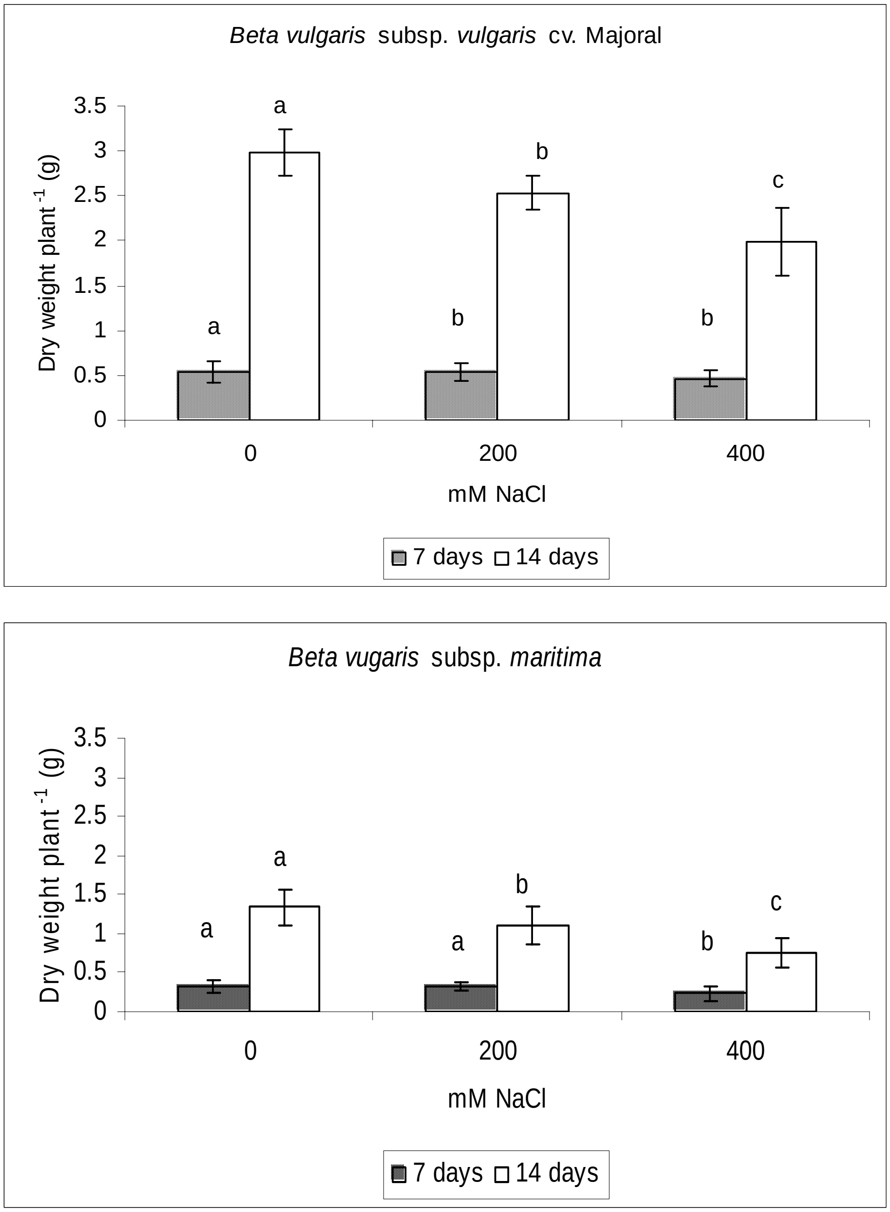


Fig 1. Effect of high salinity (200 and 400 mM NaCl) on dry weight plant^{-1} of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp *maritima*) at 7 and 14 days after salt treatments (P < 0.05) with standard error bars. Average of 10 replications.

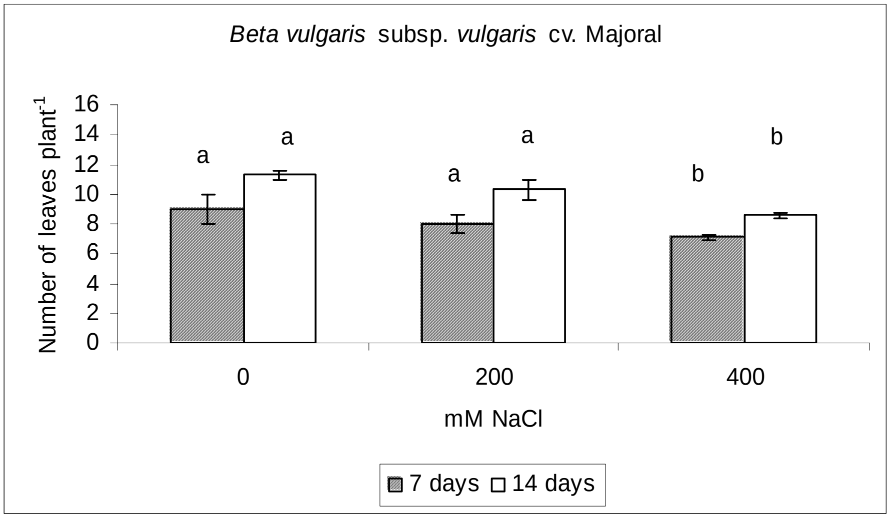
(Fig 1). In the presence of 400 mM NaCl, plant dry weight decreased significantly in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) after 14 days of growth.

The number of leaves per plant was lower in seabeet (*Beta vulgaris* subsp. *maritima*) compared to fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) in the first week. The number of leaf per plant decreased with the high salinity in the second week in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet (*Beta vulgaris* subsp. *maritima*). Figure 2 illustrate the number of leaf per plant in seabeet (*Beta vulgaris* subsp. *maritima*) that significantly increased in the second week of growth compared to fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral).

It is clear from Fig. 3 that the increase in leaf area per plant of both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) did not change at 200 and 400 mm NaCl compared to control in the first week of growth. The leaf area per plant in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) decreased with increasing salinity after two weeks of plant growth. The average leaf area per plant in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) was greater than that of seabeet (*Beta vulgaris* subsp. *maritima*). Relative growth rate (RGR, mg g^{-1} day^{-1}) increased in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) with high salinity (400 mM NaCl) after one week of salt treatment (Table 18). The RGR of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was higher than that of seabeet (*Beta vulgaris* subsp. *maritima*). The RGR significantly decreased in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) beyond 200 mM NaCl salinity after 14 days (Table 18). Net assimilation rate (NAR g m^{-2} day^{-1}) of fodderbeet (*Beta vulgaris* subsp.

vulgaris cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) increased with high salinity after first week. During the second growth week the NAR significantly decreased beyond 200 mM NaCl in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). Leaf area ratio (LAR m^2 g^{-1}) remained unchanged in salt treated fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) plants throughout the growth period, but LAR in seabeet (*Beta vulgaris* subsp. *maritima*) increased after the first week compared to the control (Table 18). The leaf weight ratio (LWR mg g^{-1}) of seabeet (*Beta vulgaris* subsp. *maritima*) was greater than fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) throughout the growth period. The LWR increased in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) with increasing salinity after one week. However, LWR of seabeet (*Beta vulgaris* subsp. *maritma*) remained non-significant during the growth period tested. The specific leaf area (SLA m^2 g^{-1}) in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) increased significantly after the second week at 200 and 400 mM NaCl.

Net photosynthesis (µmol CO_2 m^{-2} s^{-1}) significantly increased in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) with high salinity for the first week of growth. After the second week the net photosynthesis decreased significantly with high salinity (200 and 400 mM NaCl) (Table 19). There was no significant change in the rate of transpiration recorded with increasing salinity in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). However, a significant increase in transpiration was observed after the second week of growth. The water potential significantly increased with the increasing salinity in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) in the second week of growth (Table 19). After the second week, the water potential in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) increased compared to the first week, but the water potential was



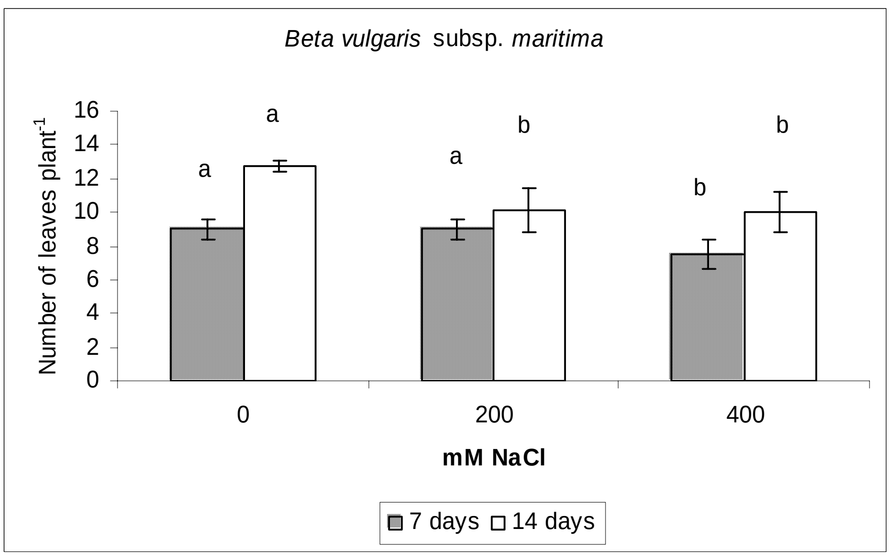


Fig 2. Effect of high salinity (200 and 400 mM NaCl) on number of leaf plant^{-1} of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and (*Beta vulgaris* subsp. *maritima*) seabeet at 7 and 14 days after salt treatment ($P < 0.05$) with standard error bars. Average of 10 replications.

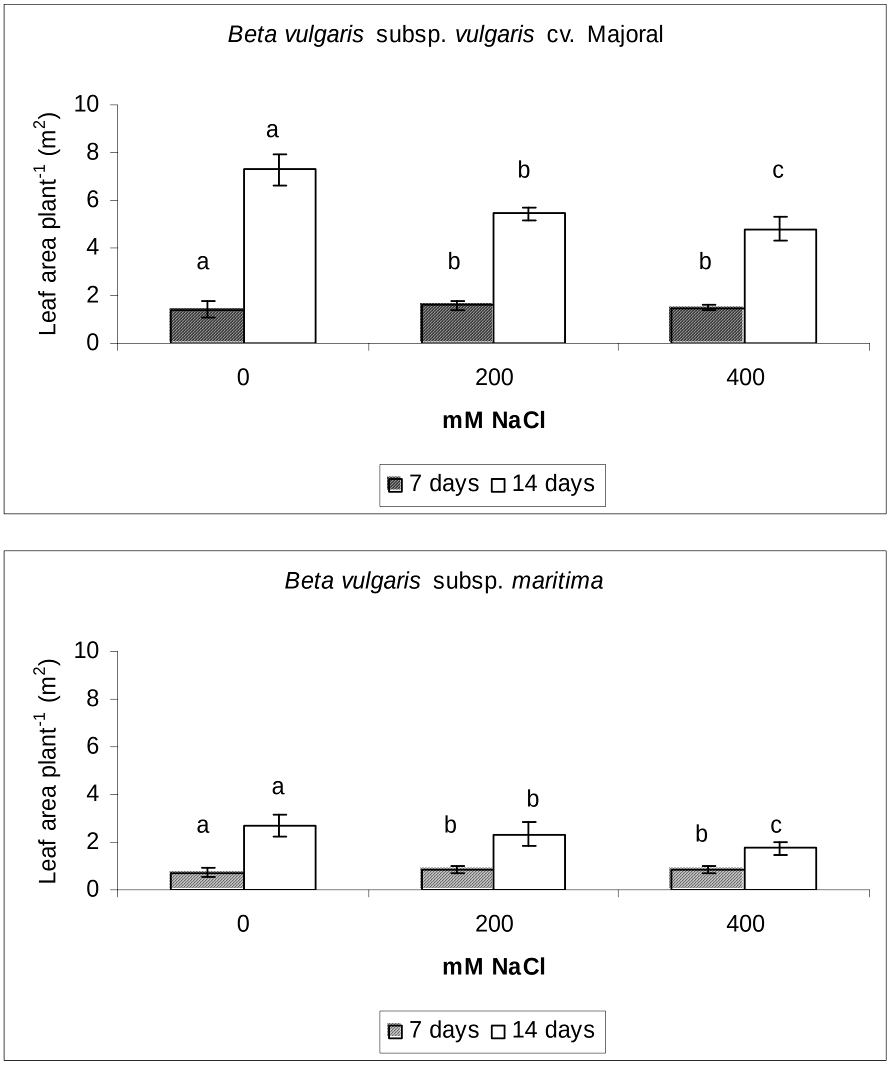


Fig 3. Effect of high salinity (200 and 400 mM NaCl) on leaf area of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp *maritima*) at 7 and 14 days after salt treatment ($P < 0.05$) with standard error bars. Averages calculated of 10 replications.

comparatively higher with increasing salinity (200 and 400 mM NaCl) after the second week in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). The osmotic potential

Table 18. Effect of different salinity levels on growth parameters of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at two growth periods after salt treatment. Averages calculated of 10 replications. One-way ANOVA for fodderbeet and seabeet was calculated per harvest (after 7 days and 14 days) for each parameter separately. Significance for salinity was computed as * = P<0.05, ** = P<0.01, ns = non significant.

	Relative growth rate (mg g^{-1} day^{-1})							
Days	7			F value	14			F value
mM NaCl	0	200	400		0	200	400	
Fodderbeet	66.81	85.5	81.21	18.7	242.1	201.7	150.8	61.5
				**				**
Seabeet	68.75	66.58	79.72	11.4	198.9	174.2	88.99	153.8
				**				**
	Net assimilation rate (g m^{-2})							
Fodderbeet	5.68	7.25	7.27	22.2	9.00	9.11	5.86	61.5
				**				**
Seabeet	6.59	5.57	7.69	30.5	8.96	7.04	3.67	179.4
				**				**
	Leaf area ratio (m^2 kg^{-1})							
Fodderbeet	12.36	11.69	12.36	2.6	14.13	15.88	14.59	4.6
				ns				ns
Seabeet	15.08	17.56	14.91	10.5	14.84	15.57	16.44	0.3
				**				ns
	Leaf weight ratio (mg g^{-1})							
Fodderbeet	698.6	794.1	758.6	4.9	673.6	603.5	628.0	3.8
				*				ns
Seabeet	819.0	861.6	795.4	2.0	689.1	765.3	724.6	3.3
				ns				ns
	Specific leaf area (cm^2 kg^{-1})							
Fodderbeet	176.9	147.2	169.3	10.5	209.7	263.2	232.3	15.5
				**				**
Seabeet	184.0	203.8	187.5	3.6	215.4	203.4	226.9	3.6
				ns				ns

Table 19: Effect of different salinity levels on physiological parameters of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) at two growth periods after salt treatments. Averages calculated of 10 replications. One-way ANOVA for fodderbeet and seabeet was calculated per harvest (after 7 days and 14 days) for each parameter separately. Significance for salinity was computed as * = P<0.05, ** = P<0.01, ns = non significant.

	Photosynthesis (µmol CO_2 m^{-2} s^{-1})							
Days	7			F value	14			F value
mM NaCl	0	200	400		0	200	400	
Fodderbeet	4.0	4.45	4.75	8.9	3.38	2.57	3.52	30.3
				*				**
Seabeet	3.54	3.71	4.09	6.5	2.39	2.14	1.81	22.0
				*				**
	Transpiration (mmol H_2O m^{-2} s^{-1})							
Fodderbeet	0.72	0.71	0.69	0.5	3.81	3.36	4.03	10.0
				ns				**
Seabeet	0.75	0.69	0.67	4.6	5.07	4.65	5.41	6.9
				ns				*
	Water potential (-bars)							
Fodderbeet	11.5	11.0	12.65	5.4	6.37	8.62	10.62	71.6
				*				**
Seabeet	10.87	11.5	11.87	2.3	9.75	11.5	9.12	17.2
				ns				**
	Osmotic potential (-bars)							
Fodderbeet	15.75	14.15	15.52	3.7	9.23	11.62	13.02	35.3
				ns				**
Seabeet	13.07	13.35	13.82	1.0	12.55	14.10	13.72	4.3
				ns				ns
	Moisture content %							
Fodderbeet	95.16	95.15	94.48	0.02	94.81	92.46	93.46	0.2
				ns				ns
Seabeet	93.20	93.43	92.98	0.01	92.50	92.57	93.13	0.02
				ns				ns

increased with increasing salinity level in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) after the second week of growth. Seabeet (*Beta* *vulgaris* subsp. *maritima*) had a significantly higher osmotic potential than fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) in all treatments after two weeks of plant growth (Table 19). The water potential significantly differed with high salinity in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*). Moisture content of the leaves in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) did not change throughout the growth period (14 days) (Table 19).

Discussion

A comparison of different growth parameters of fodderbeet cultivars and seabeet showed that RGR values in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) in experiment I were higher under high salinity (150 mM NaCl) than those in seabeet (*Beta vulgaris* subsp. *maritima*). The values of RGR were within the range of herbaceous plants mentioned by Poorter (1989). The RGR of fodderbeet and seabeet decreased significantly during the period of six weeks of experiment I. The NAR increased for *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monored in the presence of 150 mM NaCl. The NAR significantly increased in six weeks compared to the first harvest. The leaf area per plant also increased significantly during that period. The rate of photosynthesis was increased with increased salinity in *Beta vulgaris* subsp. *maritima*. An increase in SLA was associated with a decrease in LWR. It was related to a significant increase of the leaf thickness in seabeet (Table 3). Salinity affected the pre-dawn leaf water potential, stomatal conductance, evapotranspiration, leaf area and yield of the plants (Katerji *et al.*, 2003; Stewart and Dwyer, 1983). There was no significant change in the total water potential of the shoots (pressure bomb) under saline conditions except in *Beta vulgaris* subsp. *maritima*. The osmotic potential was significantly reduced (more negative) under 150 mM NaCl. In the present study a significant increase in leaf and root dry weight of *Beta vulgaris* subsp. *maritima* was recorded with increased salinity (150 mM NaCl) in the first two weeks, while in the *Beta vulgaris* subsp. *vulgaris* cv. Majoral, a reduction in the dry weight of leaves and roots was observed under saline conditions (Table 4). A lower number of leaves per plant in fodderbeet cultivars compared to seabeet under high salinity may correlate to reduced dry weight of shoot biomass (Table 5). *Beta vulgaris* subsp. *maritima* had the highest average number of leaves, but the total leaf area per plant was less compared to the fodderbeet cultivars due to a smaller leaf area of the individual leaf. The leaves of *Beta vulgaris* subsp. *maritima* were more fleshy.

The age of fodderbeet plants may have caused an interaction between cultivars and the salinity treatment. Fodderbeet plants possibly used up most of the energy available for ion pumps involved in compartmentation and secretion and repair of cellular damage caused by the saline conditions (Penning de Vries, 1975; Schwarz and Gale, 1981) to overcome the effect of salinity. Also the hormonal and physical (i.e. turgor potential) factors controlling cell wall loosening and cell expansion (Cleland, 1986) may have adjusted during the growth period of the fodderbeet. The amount of photosynthesized material translocated to roots under saline conditions was relatively low during the first two weeks. After four weeks a considerable amount of dry matter accumulated in the root system (Table 4). This adaptation may be related to increase in leaf area with high salinity (*Beta vulgaris* subsp. *maritima*, +13.8 %) (Table 4). The fodderbeet (*Beta vulgaris* subsp. *vulgaris* cultivars) and seabeet (*Beta vulgaris* subsp. *maritima*) appeared to differ with respect to LAR and LWR. The difference may be related to the significantly negative effect of salinity treatment on SLA in all except *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia cultivars and seabeet *Beta vulgaris* subsp. *maritima* (Table 2).

Beta vulgaris subsp. *maritima* is the halophytic ancestor of fodderbeet growing in coastal areas of the Atlantic and Mediterranean, while the fodderbeet cultivars are its domesticated breeds. The wild *Beta vulgaris* subsp. *maritima* has a rosette structure, a greater number of leaves and comparatively narrower leaves than the fodderbeet cultivars. The leaves of the fodderbeet cultivars are broader and have a significantly larger leaf area. They intercept more light and synthesize more biomass per leaf than seabeet. This may underly the larger RGR of the fodderbeet cultivars compared to seabeet. Similar findings had also been discussed by Rozema (1990; 1991) and Rozema *et al*. (1993).

Experiment II

The biomass accumulation of fodderbeet and seabeet shoots in experiment II did not change during the first two weeks with increasing salinity (200 mM NaCl). After two weeks the plants may have adapted to the saline environment and the increase in the biomass of shoot was significant with the high salinity compared to the control treatment (0 mM NaCl). The dry weight accumulation of the fodderbeet and seabeet during the first two weeks was limited but for the last two weeks (21-35 days) accumulation of dry matter was significantly higher at 200 mM NaCl compared to 0 mM NaCl. This confirms that the plants had adapted to the saline environment after the two weeks growth period. The fresh weight of the roots increased in the control treatment compared to 200 mM NaCl after the fourth week (35 days). Fresh and dry weights of the roots of fodderbeet were significantly higher than that of seabeet. The roots of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) consist of a tap root system, without any branches, while those of seabeet (*Beta vulgaris* subsp. *maritima*) are a branched tap root system. The difference in the structure of the roots of both plants may have resulted in a difference in the biomass accumulation**.** The fresh and dry weight ratio of shoots and roots in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) were higher than that of seabeet (*Beta vulgaris* subsp. *maritima*) during the growth period studied (7-35 days). It showed increased biomass accumulation in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) roots compared to those of seabeet (*Beta vulgaris* subsp. *maritima*).

The presence of a higher concentration of NaCl (200 mM NaCl) in the growth medium increased the concentrations of Na^+ by the plant. They absorbed more Na^+ under saline conditions from the salinized growth medium than from the control medium the Na^+ concentration of the plant tissue increased during growth until the final harvest. Movement of ions from roots to the shoots with increased salinity was higher than that at control. Different plant species differ in the underlying mechanism of Na^+ influx to the plants. If a root extrudes Na^+ it is not going away, not in the soil at any rate, where the mass flow of solution is predominantly toward the root and much, much greater than diffusion away from it. There will be advection of salt towards the roots because the mass flow of the solution is partitioned at the root surface, the water being taken up and the salt left behind. A good discussion in this regard had also been documented by Yadev *et al*., (1996), Tyerman *et al*., (1997) and Yeo (1998).

The higher Na^+ and Cl^- ion accumulation by shoots and leaves of *Beta vulgaris* subsp. *maritima* confirm the physiological status of many members of the family Chenopodiaceae as salt accumulators (Rozema *et al*., 1981). Salt accumulation is considered to be one of the indices of salt tolerance (Shannon and Grieve, 1999). There was increased Cl^- ion accumulation in the roots up to the third week with increased salinity. The concentrations of chloride further increased in seabeet (*Beta vulgaris* subsp. *maritima*) until the final harvest in the presence of a high NaCl concentration. A highly significant correlation between Na^+ and Cl^- ions was recorded in the shoots and roots of both the fodderbeet and seabeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral, r^2 = 0.93 and *Beta vulgaris* subsp. *maritima*, r^2 = 0.97 respectively). The salinity (200 mM NaCl) induced growth reduction in *Beta vulgaris* subsp. *maritima* was lower than in fodderbeet cultivars (Niazi *et al*, 1999) which is an indication of their high salt tolerance. This may be related to a lower chloride concentration in shoot of *Beta vulgaris* subsp. *maritima* compared to that of *Beta vulgaris* subsp. *vulgaris* cv. Majoral (Table 10). *Beta vulgaris* subsp. *maritima* tolerated a salinity level up to 120 mM NaCl in a nutrient solution study (Smolders and McLaughlin, 1996a). In an outdoor sand culture experiment at the U.S. Salinity Lab, an increase in the dry weight of seabeet was reported up to 11 dS m^{-1} (~ 110 mM NaCl) above which the dry weight further reduced at a rate of about 5.7 % per dS m^{-1} (Shannon and Grieve, 1999). The calculated 50 % decrease in the dry weight compared to the control for seabeet yield was at an EC of 19.8 dS m^{-1}. Similarly Osawa (1996) reported 50 % decrease in the dry weight of *Beta vulgaris* subsp. *maritima* at 17.5 dS m^{-1} compared to the control. In the present study the dry weight of the shoots significantly increased in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) after 35 days of plant growth, while the dry weight of the roots in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) significantly decreased. The salinity level of 200 mM NaCl ~ 20 dS m^{-1} was slightly higher than the threshold salinity level reported by Osawa (1996).

A negative correlation (r^2 = -0.77) was observed in the Na shoot/K root ratio in *Beta vulgaris* subsp. *vulgaris* cv. Majoral while *Beta vulgaris* subsp. *maritima* showed a stronger negative correlation value (r^2 = -0.88) than that of fodderbeet (correlation values calculated from the data in Tables 9 and 11). Niazi and Ahmad (1984) have also confirmed an antagonistic effect between Na^+ and K^+ ions during a study on the uptake of ions in tomato under saline soil conditions. Generally low K^+ uptake by the roots is believed to involve inward-rectifying K^+ channels, allowing K^+ to enter along an electrochemical gradient (Smart *et al*., 1996; Miedema, *et al.*, 2003). This probability does not mediate K^+ uptake by roots, as it is expressed predominantly in leaf tissue in leaf guard cells and vascular tissue of stem and root, and has been proposed as a mechanism of low affinity uptake into guard cells (Nakamura *et al*., 1995). As was discussed earlier, the majority of plants tend to exclude Na^+ from the shoots by retaining it in the roots and by maintaining a low concentration in the stems. This strategy is only successful at low to moderate external concentrations of Na^+ and relies on the selective release of Na^+ into the xylem and its re-sorption from the xylem stream. Similar suggestions have also been made by Frans *et al*., (1999), Fu and Luan (1998), Hirch *et al*., (1998), Amtmann and Sanders (1999), Maathuis *et al*, (1996) and Tyerman and Skerrett (1999).

Some grass species growing in nutrient cultures containing NaCl as a sole osmoticum, frequently displayed leaf blade deformation and necrosis that are characteristic of severe Ca-deficiency (Kawasaki and Moritsugu, 1979). The physiological role of Ca^{2+} in the cellular system is to stabilize the cell wall structure in order to maintain membrane integrity, selectivity and to regulate selectivity of ion transport process (Hanson, 1983). Membrane permeability may increase by Na^+ -displaced Ca^{2+} ions that are associated with the external surface of the plasmalemma (Cramer *et al*., 1985). In the present study an increase in Ca^{2+} concentration of the roots was observed in the presence of NaCl. This may form part of a highly salt tolerant behaviour of the plants. The magnesium concentration did not decrease with age in *Beta vulgaris* subsp. *vulgaris* cv. Majoral, and in *Beta vulgaris* subsp. *maritima* even an increase in Mg^{2+} was observed at 200 mM NaCl. The correlation between Na^{+} roots and Mg^{2+} shoots was significantly negative (r^2 = -0.77) in *Beta vulgaris* subsp. *vulgaris* cv. Majoral, while this same correlation was highly positive (r^2 = 0.91) in *Beta vulgaris* subsp. *maritima* (correlation values calculated from the data in Tables 9 and 13). An increase of Ca^{2+} in the leaf may result in a marked reduction in leaf Mg^{2+} (Bernstein and Hayward, 1958). This Ca^{2+}- Mg^{2+} antagonism could lead to a disturbance in photosynthesis. Reduced photosynthesis was noted in maize due to Mg^{2+} deficiencies (Peaslee and Moos, 1966). High Ca^{2+} in leaf may also interfere with CO_2 fixation by inhibition of stroma enzymes especially those that are Mg activated (Charles and Halliwell, 1980). The present study proved no disturbance in Ca^{2+}- Mg^{2+} imbalance and Mg^{2+} tended to increase with simultaneous increase in Ca^{2+} concentration. This may result in a better net photosynthesis of fodderbeet and seabeet in the presence of high NaCl (Niazi *et al*., 1999). This may be an explanation for the observed salt tolerance of fodderbeet and seabeet. Similar conclusions were suggested by Knight *et al*. (1997), Davenport *et al*. (1997), Liu and Zhu (1997; 1998), Kinraide (1998) and Bressan *et al*. (1998).

During the onset and development of salt stress within a plant all the major processes such as photosynthesis, protein synthesis and energy and lipid matabolisms are affected (Parida and Das, 2005). Our results indicate that the chlorophyll concentration in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) remained unaffected with increasing salinity, while in seabeet (*Beta* *vulgaris* subsp. *maritima*) the chlorophyll 'a' and 'b' concentrations were significantly changed with increasing salinity (Table 14). However, due to the comparatively larger leaf area in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) more chlorophyll molecules were exposed to light than that in seabeet (*Beta vulgaris* subsp. *maritima).* The light energy interception in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) due to the larger leaf area per plant will have been greater than that of seabeet (*Beta vulgaris* subsp. *maritima*). An important factor that controls growth, and especially dry matter accumulation, is the photosynthetic activity of the plant. The role of photosynthesis does not only determine accumulation of plant structural and storage material, but the products of photosynthesis may also be important for osmoregulation. It may therefore be expected that photosynthesis may show lesser sensitivity to salinity in salt tolerant species in which growth and yield are less inhibited by salinity. Hence the synthesis of sugar may have been higher. It is known that some halophytic plants (*Atriplex amnicola*) need more energy under saline soil conditions (Aslam *et al*., 1986). Extra energy could be provided by the increased content of sugars which are energy-rich compounds. Lower water-soluble sugar concentrations in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) as compared to sugarbeet have been reported (Quin *et al*., 1980). In the present study the sugar concentrations decreased in both plant sub-species with higher salinity (Table 15). This may be related to the increased Mg^{2+} concentrations (Niazi *et al*., 1999), because of its importance as a co-factor in almost all enzyme-activated phosphorylating processes. Magnesium is also required for the activation of ribulose 1,5-bisphosphate carboxylase (Bassham, 1979) and in carbon dioxide assimilation and related processes, such as the production of sugar and starch (Greger and Linberg, 1987). The sugar concentrations in this experiment are also increased after addition of Cl^- along with roots fresh and dry weights

(Table 15). An increase in the K^+ uptake was reported by Niazi *et al*. (2004) and may increase the translocation of photoassimilates from leaves to roots, thus increasing the amount of root dry matter (Hartt, 1970; Magat and Goh, 1988). There was no increased uptake of K^+ noted in the present study. Maximization of root yield has also been related to the nitrogen content that lead to lower sucrose concentration in the roots (Anderson and Peterson, 1988). In the present study nitrogenous fertilizer was not added to the pots. Therefore, increase in sugar concentrations may be related to the increased concentrations of Mg^{2+} in seabeet (*Beta vulgaris* subsp. *maritima*) under high salinity (Table 13).

A decrease in the total protein concentrations of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) roots during the second and third week of growth may be due to translocation of proteins to the shoots. This is confirmed by a consequent increase of protein in the shoots until the fourth week. There was a significant increase in the protein concentrations of the seabeet (*Beta vulgaris* subsp. *maritima*) shoots under increasing salinity. An increase in the sugarbeet protein content due to micronutrient nutrition has been reported by Firgany *et al*., (1981); Draycott and Bugg, (1982). The protein concentrations in the leaves have also been shown to increase under salinity by Haeder and Mengel (1972) and this is confirmed by the present study.

Compatible osmolyte (Glycinebetaine)

Various metabolic and defence systems are activated in the plants experiencing environmental stresses like drought, high salinity and low temperatures. A number of genes corresponding to these stresses and their products were analysed in *Arabidopsis* (Oono *et al*., 2003; Maruyama *et al*., 2004) and rice (Rabbani *et al*., 2003). Osmoprotectants, such as proline, glycinebetaine, manitol and sugars confer stress tolerance (Taji *et al*., 2002; Abebe *et al*., 2003; Yamada *et al*., 2005). Wyn-Jones and Storey (1981) reported that the accumulation of glycinebetaine is a good indicator of salt tolerance in different plants. Glycinebetaine is very mobile (Mc-Donnel and Wyn-Jones, 1988). Betaine increased with increasing K^+ concentrations in storage roots of sugarbeet (Beringer *et al*., 1986), which may relate to high glycinebetaine levels as observed in our present study (Table 17). The fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) plants showed a considerable salt tolerance during the two weeks vegetative growth period (Experiment II) under saline soil conditions in the present study. The mechanism of salt tolerance may in part be achieved through accumulation of more K^+, Ca^{2+} and Mg^{2+} ions. Sequestration of Na^+ ions into the cell vacuole is one of the mechanisms to confer Na^+ tolerance on *Arabidopsis thaliana*. The genes are being identified in low affinity electro-neutral Na^+/H^+ exchangers (Darley *et al*., 2000). Several proteins have been characterized that play a prominent role in the regulation of K^+ and /or Na^+ fluxes (Maathuis and Amtmann, 1999). These ions may help to improve the synthesis of sucrose and protein, which activate the biosynthesis of glycinebetaine, thus increasing the salt tolerance of plants (Yamada *et al*., 2005; Rabbani *et al*., 2003). Seabeet (*Beta vulgaris* subsp. *maritima*) is a halophyte plant, growing in the saline coastal areas. The aerial part of seabeet (*Beta vulgaris* subsp. *maritima*) is extensively branched and the number of leaves exceeds that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). However, it is clear from table 4 that the leaf area per plant of seabeet (*Beta vulgaris* subsp. *maritima*) is less than that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) (Table 4). Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) is the domesticated cultivar originating from seabeet (*Beta vulgaris* subsp. *maritima*) with a larger leaf area per plant, which helps the plant in the interception of sunlight. This will account for the accumulation of a higher concentration of sugar and glycinebetaine resulting in the higher energy storage by the plants. In the seabeet (*Beta vulgaris* subsp. *maritima*), higher chlorophyll concentrations present at the fifth week of growth period (Table 14) could not help the accumulation of plant biomass effectively because of the smaller leaf area compared to that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). The larger leaf size in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) may have successfully contributed to increased carbohydrate concentrations by intercepting more sunlight. Consequently, this may have provided extra energy to overcome salt stress experienced by the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plant.

In Experiment III the dry weight per plant accumulation in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* did not increase with increasing salinity (200 and 400 mM NaCl) compared to control (0 mM NaCl). This indicated that the plants may have recovered the transplantation shock and were adjusted to high salinity during the first 2 weeks of growth. During the later growth period the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* decreased significantly with the high salinity (200 and 400 mM NaCl) compared to the control. The dry weight accumulation per plant was however greater in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral compared to seabeet *Beta vulgaris* subsp. *maritima* (Fig 1). The average leaf area per plant in *Beta vulgaris* subsp. *vulgaris* cv. Majoral was significantly higher than that of *Beta vulgaris* subsp. *maritima*. The leaf area significantly decreased with increasing salinity (200 and 400 mM NaCl) compared to the control treatment (Fig 3).

The number and arrangement of leaves in the two cultivars was different (Fig 2). Fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral had a lower number of leaves, with larger individual leaf area per plant and reduced stem length, while seabeet *Beta vulgaris* subsp. *maritima* had a larger leaf number with a smaller individual leaf area and a rosette arrangement of leaves. This may reflect a difference in radiation interception resulting in different dry weight accumulation (Martin, 1986).

The relative growth rate decreased under increasing saline conditions (400 mM NaCl) in both fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima*. After two weeks a decrease in RGR may be related to a significant decrease in leaf area per plant with increasing salinity during the same growth period in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima*. Net assimilation rate increased or decreased relating to the RGR of respective species. A significant decrease in LAR and a decrease in SLA under saline conditions (200 mM NaCl) for seabeet *Beta vulgaris* subsp. *maritima* (Table 18) may be related to an increase in leaf thickness (Niazi *et al*., 1995). The leaf area ratio significantly increased during the plant growth. The reduction in LAR may also be related to the attainment of a greater leaf size in *Beta vulgaris* subsp. *vulgaris* cv. Majoral. *Beta vulgaris* subsp. *maritima* showed a smaller reduction in LAR with increasing salinity (400 mM NaCl) during the first week. The leaf area of *Beta vulgaris* subsp. *maritima* was smaller than that of *Beta vulgaris* subsp. *vulgaris* cv. Majoral. A significantly higher number of leaves in *Beta vulgaris* subsp. *maritima* than in *Beta vulgaris* subsp. *vulgaris* cv. Majoral may play an important role in the difference in LAR. The presence of a higher number of leaves per plant leads to a specific pattern for expansion in which the size of successive leaves is decreased (Milford *et al*., 1985). The area of the individual leaves in *Beta vulgaris* subsp. *maritima* was relatively small. Leaves of *Beta vulgaris* subsp. *maritima* were more succulent than those of *Beta vulgaris* subsp. *vulgaris* cv. Majoral at 400 mM NaCl (Niazi *et al*., 1995). The specific leaf area of *Beta vulgaris* subsp. *maritima* was less at higher salinity (200 and 400 mM NaCl) in the second week, than that of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral when compared to the respective harvests. This supports the findings that *Beta vulgaris* subsp. *maritima* tended to have more succulent leaves with a higher number of mesophyll tissue layers and intercepted greater light energy than the other cultivars. Therefore, NAR of the plants did not decrease with increasing salinity up to 200 mM NaCl (Table 2). Hence the adjustment of the seabeet *Beta vulgaris* subsp. *maritima* to salinity may be related to the activity of the physiological components of plant growth (NAR) under saline conditions (Fig 1, chapter 1). The *Beta vulgaris* subsp. *vulgaris* cv. Majoral had a higher leaf dry weight because of its greater average leaf area per plant, which may relate to a greater LAR. The increase in the LWR with high salinity after one week of plant growth in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral may relate to improvement in the morphological component of growth for salt tolerance of the plants.

Net photosynthesis in neither the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral nor seabeet *Beta vulgaris* subsp. *maritima* was affected under saline conditions after 7 days. *Beta vulgaris* subsp. *vulgaris* cv. Majoral had a higher rate of photosynthesis than seabeet *Beta vulgaris* subsp. *maritima*. There was an increase in photosynthesis after 14 days at 400 mM NaCl in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral.

Salinity did not affect the transpiration rate (Table 19), which indicates that there is no salinity-induced limitation on the function of the stomata. Unaffected transpiration and undisturbed water uptake probably did not only help but may have produced a positive effect on cell wall surface extension and cell growth as well (Cleland 1986), which relates to a positive linear increase in whole plant dry weight, even at a high salinity level (200 mM NaCl).

Fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral produced a significantly greater biomass than seabeet *Beta vulgaris* subsp. *maritima* under saline conditions (150 mM NaCl) (Experiment I). The biomass production of *Beta vulgaris* subsp. *vulgaris* cv. Majoral was related to a higher average leaf area per plant during the later growth period. A significantly higher synthesis of osmolyte (glycinebetaine and proline) may have helped the plant to tolerate the high salinity. The rate of transpiration and photosynthesis was generally not affected by high salinity (200 and 400 mM NaCl) which was related to a higher NAR, and thus the growth rate of the plants increased. The fleshy leaf of *Beta vulgaris* subsp. *maritima* showed a trend toward increased leaf thickness with increasing salinity that was also observed in the solution culture experiment with increasing salinity of 200 and 400 mM NaCl (chapter 3). Therefore, the salt tolerant fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral appears to be suitable for cultivation in salt-affected areas of developing countries like Pakistan.

Fodderbeet is halophytic in nature and may tolerate salinity of 200 mM. The seabeet (*Beta vulgaris* subsp. *maritima*) is salt tolerant growing in the coastal places in Europe and around the Mediterranean. The domesticated cultivars of fodderbeet including *Beta vulgaris* subsp. *vulgaris* cv. Majoral have been tested in the inland areas of Pakistan (Niazi *et al*., 1999) as described in chapter 5. The salt-affected areas in Pakistan could be utilized by cultivation of these fodderbeet cultivars to increase the production of fodder for cattle as well as utilization of unproductive soils in the resource poor countries like Pakistan.

CHAPTER 5

Chapter 5

Fodderbeet Grown on Saline-sodic Soils of Pakistan

Abstract

The results obtained from three field experiments in Pakistan are presented in this chapter. In the first experiment plant growth of four fodderbeet cultivars was compared in non-saline and saline-sodic soils under field conditions. The pH and EC_e of the saline-sodic soil decreased at the time of harvest compared to that of pre-sowing of fodderbeet crop. The fresh weight of the beet in the fodderbeet cultivars (*Beta vulgaris* subsp. *vulgaris* cvs. Monored and Majoral) increased significantly, under saline soil compared to non-saline soil conditions. The fresh weight of fodderbeet cultivars (*Beta vulgaris* subsp. *vulgaris* cvs. Monoval and Polygroeningia) however, decreased under saline soil conditions. The fresh weight of the leaves in all the tested cultivars increased significantly under the saline soil conditions. The chlorophyll ‘a’ and chlorophyll ‘b’ concentrations of the leaves in all the four cultivars increased under the saline soil conditions. The highest chlorophyll a/b ratio was recorded in the *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia. The sugar concentrations of the beet in all the cultivars increased under saline soil conditions. However, the sugar concentrations of the leaves decreased in *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monoval, while sugar concentrations in the leaves of *Beta vulgaris* subsp. *vulgaris* cvs. Monored and Polygroeningia remained unchanged. The protein and proline concentrations of the beet and leaf of all the cultivars was increased under saline soil conditions. The Pakistan farmers grow conventional fodder crops (barley and oat) in the area to feed the cattle. A second experiment was designed to compare the biomass production of three fodder crops i.e. two conventional fodder crops barley (*Hordeum*

vulgare) and oat (*Avena fatua*), and a non-conventional crop (fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral) in a saline-sodic field. The number of leaves in fodderbeet was significantly higher than that in barley and in oat. The leaf area per plant of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was about two times higher compared to that of tested conventional crops. The chlorophyll concentrations in fodderbeet were double than that of barley and oat. The fresh weight (whole plant) of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was three times higher than that of the conventional crops. The comparatively higher biomass increase in fodderbeet may relate to the weight of the beet, which contributed considerably to the fresh weight of the plants.

Farm yard manure (FYM) is used to supplement the soil fertility by the farmers in Pakistan. However, use of farm yard manure is not common in the salt-affected soils. The response of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral in a farm yard manure-amended saline-sodic soil was studied in a pot experiment. The experiment was conducted on a normal non-saline, non- sodic soil (pH = 7.62, EC_e = 1.07 dS m^{-1}, ESP = 1.01) and on a saline-sodic soil (pH = 9.5, EC_e = 7.9 dS m^{-1}, ESP = 51.73). The effect of farm yard manure amendment on the growth of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) produced the following results. Addition of FYM to non-saline soil increased the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoot and root, number of leaves, leaf area per plant, protein concentrations and chlorophyll concentrations. Fresh and dry weight and the number of leaves per plant increased significantly in the presence of FYM. The protein and sugar concentrations increased in the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral root but the chlorophyll 'a' concentrations of the leaves remained unaffected with high salinity.

In the first field experiment, comparison of the growth of four fodderbeet (*Beta vulgaris* subsp. *vulgaris*) cultivars under saline-sodic soil relates to decrease in the pH and EC of the saline-sodic soil. The growth of fodderbeet cultivars increased under saline-sodic soil conditions. The growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was comparatively greater than other cultivars tested. in the second field experiment, the biomass, number of leaves, leaf area per plant and chlorophyll concentrations of non-conventional fodder crop (Fodderbeet, *Beta vulgaris* subsp. *vulgaris* cv. Majoral) increased under saline-sodic soil conditions compared to that of conventional fodder crops (barley and oat). In the third (pot) experiment FYM added to non-saline soil improved the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral while growth of the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral decreased under FYM-amended saline-sodic soil.

Introduction

Salinity and temperature stresses are primary limiting environmental conditions significantly restricting the successful cultivation of crops in irrigated arid and semi-arid regions (Bergmeyer and Brent, 1974). The area of salt-affected non-arable land is increasing in the region irrigated with canal water in North West Frontier Province (N. W. F. P.), Pakistan. The increasing soil salinity is due to the rise in the water table resulting from the seepage from canal beds. Currently about 11.98 million ha of land is under moderate to high salinity/sodicity (Muhammad, 1990). The production of almost every conventional crop in this area is significantly reduced under saline soil conditions. Hence the introduction of non-conventional salinity-tolerant crops could be a suitable option. Fodderbeet was reported to be salt tolerant (200 mM NaCl) during the vegetative growth stage (Niazi *et al*., 1997). It is extensively grown in the coastal areas in many European countries and New Zealand (Furunes, 1988; Goh and Magat, 1989). Fodderbeet requires NaCl for better growth and biomass (Draycott and Bugg, 1982; Magat and Goh, 1988).

Nutrients such as inorganic N, P, K may become unavailable in salt-affected sodic soils as a result of physico-chemical relationship (Table 1). Phosphates added as fertilizer to some soils of Pakistan immobilize the nutrients already present in the soil (Sharif, *et al*., 1966). The crops utilize only 15 to 20% of the applied phosphate and the rest is not readily available (More and Ghonsikar, 1988). The availability of nutrients by the plant under saline-sodic soil conditions is therefore limited. Addition of farm yard manure (FYM) to the soil increases the availability of nutrients to the plant (Sharif, *et al*., 1974). Singh and Dubey (1987) have reported that among other nitrogen sources, FYM has a beneficial effect on wheat production. Organic manure has a dual effect on the improvement of the soil environment. It not only increases the nitrifying activities of micro-organisms but nitrogen is also lost by increasing the cation exchange capacity of the soil (Gasser, 1964). Tisdale, *et al*. (1985) have noted a slight decrease in soil pH due to the formation of humic acids, which reduced the volatilization loss of NH_3. Salt-affected soils may be used after reclamation with chemical and organic amendments (Chaudhary, *et al*., 2004). In the past, FYM was used as an effective organic fertilizer by the farmers. The refuse of the animal was dumped and left to partly decompose, which transformed it into farm yard manure. Later with the introduction of mechanical farming and the extensive use of chemical fertilizers, the use of organic manure was reduced.

A number of experiments were conducted on the use of FYM as an amendment to the salt-affected soils. Most of the reports about the use of FYM for improved crop production contain data for alkaline calcareous soils of Pakistan (Sharif *et al*., 1966; Sharif *et al.*, 1974; Gilani *et al*., 1983; Mian, *et al*., 1989; Sandhu *et al*., 1989). However, most of the salt-affected soils in Pakistan are saline-sodic (Qadir *et al.,* 2001) and application of FYM to these soils produced improved growth of plants in these soils. Addition of nitrogen supplied to these plants in the form of FYM may help in the synthesis of proteins and osmolytes like glycinebetain and proline (Galloway and Davidson, 1993). It may be hypothesized that FYM application may induce osmolyte production with increased salt tolerance as a result.

The growth of fodderbeet in the sodic soils is reduced due to the high exchangeable sodium percentage (ESP) of the salt-affected soils. However, organic amendment with farmyard manure, poultry wastes, and compost are known to ameliorative chemical, physical and biological properties of moderately sodic soils (Ahmed *et al*., 1988).

Materials and Methods

In a field experiment (I), seed of four fodderbeet (*Beta vulgaris* subsp. *vulgaris*) cultivars viz: Monored, Monoval, Majoral and Polygroeningia were received from Holland through the courtesy of Department of Ecology and Ecotoxicology, Free University, Amsterdam. Fodderbeet *Beta vulgaris* subsp. *vulgaris* (Nomenclature according to Van der Meijden *et al*., 1990) has been identified as salt tolerant by Rozema (1991). For the layout of field experiments two sites (a non-saline and a saline-sodic field) were selected at the Arid Zone Research Sub-station (AZRSS), Dera Ismail Khan, Pakistan (Fig 2, chapter 1).

Seeds were sown in black polyethylene bags filled with garden soil for raising nursery. Two-week-old fodderbeet *Beta vulgaris* subsp. *vulgaris cultivars* were transplanted in the field. Physico-chemical characteristics of experimental fields are given in Table 1. Plots measuring 6 x 4 m^2 were prepared for sowing in a Randomized Complete Block Design with five replications. Experiments were conducted during November to May at both sites for three years (1995-1997). The fields were irrigated with canal water. Recommended doses of fertilizer for the sugarbeet (Nitrogen (N) = 120 kg ha^{-1} as urea and Phosphorus (P) = 100 kg ha^{-1} as single super phosphate) were applied. Two third of the N was added at the time of transplantation, while the remaining one third was applied 6 weeks after transplantation. The fodderbeet *Beta vulgaris* subsp. *vulgaris cultivars* growth data were recorded at the time of harvest (16 weeks after transplanting). Biomass production and some physiological parameters were measured to assess the response of fodderbeet cultivars to saline-sodic conditions in Pakistan soils.

Plant material was analyzed for chlorophyll. The fodderbeet leaf samples (50 mg) were crushed with quartz sand using 2.5 ml of 80 % acetone. The samples were put into the dark (ice cold) for 3 minutes and centrifuged for 10 minutes at 4000 rpm and the volume was raised to 5 ml (Knudson *et al.*, 1977). Total proteins (Peterson, 1984) and total soluble sugars were extracted by heating 100 mg dry plant material with 10 ml deionized water for 2 hours at 90 ºC in a water bath and filtered (Bergmeyer and Brent 1974). Data obtained were subjected to Analysis of Variance test (one-way ANOVA per fodderbeet cultivar per factor) according to Little and Hills (1978). One-way ANOVA for growth parameters and biochemical analysis of fodderbeet cultivars was also computed.

Table 1. Physico-chemical characteristics of original soil (soil saturation extract) used during the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral experiment at the Arid Zone Research Sub-station, D. I. Khan (N.W.F.P). Average of three replications of a compound sample taken from non-saline soil and saline-sodic soil field. ESP= Exchangeable Sodium Percentage

Soil properties	**Non-saline soil**	**Saline-sodic soil**
pH	7.9	8.2
EC_e dS m^{-1}	2.7	11.4
CO_3^{2-} mM	Nil	Nil
HCO_3^- mM	5.1	10.9
Cl^- mM	8.5	30.1
Ca^{2+} + Mg^{2+} mM	3.0	9.4
Na^+ mM	19.0	90.6
K^+ mM	1.4	9.7
ESP	1.6	37.7
Soil Texture	Silty Clay	Silty Clay

In a second field experiment (II) conducted at the same site as Experiment I, the fields were separately prepared to compare the biomass production for cultivation of two conventional fodder crops i.e. barley and oat and a non-conventional fodder crop *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Barley (*Hordeum vulgare*) and oat (Avena fatua) were grown from seed by sowing in 4 x 4 m^2 plots with three replications. Seed of barley and oat was sown according to the rate recommended by the Department of Agriculture, North West Frontier Province. Fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral seeds were sown directly on raised beds with a plant-to-plant distance of 45 cm and a row-to-row distance of 60 cm. A uniform fertilizer dose was applied at a rate of 120, 60 and 60 kg ha^{-1} of nitrogen as urea, phosphorus as single super phosphate (SSP) and potassium as sulphate of potash, respectively. Comparison of the biomass, number of leaves, leaf area and chlorophyll concentration were made between non-conventional (fodderbeet) and conventional (barley and oat) fodder crops.

Table 2. Physico-chemical characteristics of soil in experimental fields at D.I. Khan. Three soil samples were taken from field at a depth of 0-15 cm. The samples were thoroughly mixed and a compound sample was thus analyzed for each plot. SAR = Sodium Adsorption Ratio, ESP = Exchangeable sodium percentage.

Soil parameters	**Unit**	**Site for fodder evaluation saline-sodic soil**	**Site for manorial trial**	
			Non-saline soil	**Saline-sodic soil**
Soil texture		Silty clay	Clay loam	Clay loam
pH_s	-	8.5	7.6	9.5
EC_e	$dS\ m^{-1}$	13.5	1.1	6.8
CO_3^{2-}	$meq\ l^{-1}$	Nil	Nil	4.0
HCO_3^-	$meq\ l^{-1}$	12.2	2.5	12.0
Cl^-	$meq\ l^{-1}$	25.7	7.5	280.0
$Ca^{2+} + Mg^{2+}$	$meq\ l^{-1}$	8.5	13.5	3.2
Na^+	$meq\ l^{-1}$	94.9	4.0	93.0
K^+	$meq\ l^{-1}$	7.3	2.0	0.8
SAR	-	41.0	1.5	73.8
ESP	-	37.2	1.0	51.7

The fertilizer dose was applied to the fodderbeet at transplanting and oat and barley were fertilized at the time of seed-bed preparation (conventional practice). The field was irrigated three times during the growing period. Plants were harvested 16 weeks after transplantation of fodderbeet seedlings.

Later, a third (III) experiment was conducted at the National Agricultural Research Center, Islamabad, Pakistan, to assess the performance of the non-conventional fodderbeet crop for production of biomass using farm yard manure (FYM) as an amendment in the saline-sodic soils (Table 3). Normal (non-saline, non-sodic) soil was collected from the field area of National Agriculture Research Centre, Islamabad, Pakistan. While saline-sodic soil was collected from the Saline Agriculture Sub-station, Sadhuke, Lahore (Table 3). Both soil types were air-dried, ground and sieved using a 0.5 mesh size. Each soil was divided into two halves and separately mixed with farm yard manure at the rate of 10 t ha^{-1} on fresh weight basis. The farm yard manure (FYM) used in this experiment contained buffalo and cow dung, liquid animal excreta, and organic matter including rice and wheat straw. This FYM contained 1.24% nitrogen, 0.75% phosphorus and 1.07% potassium on a dry weight basis. The amelioration of soils with FYM, produced four types of soil mixtures: normal soil without FYM, normal soil mixed with FYM, saline-sodic soil without FYM and saline-sodic soil mixed with FYM. Undrained plastic pots were thoroughly washed with tap water and dried. The pots were divided into four groups. Each group consisted of 10 pots. The pots were filled with 5 kg soil per pot from four different soil mixtures. Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) seeds were sown in plastic tubs filled with garden soil for raising the nursery. Four-week-old seedlings were transplanted to each pot containing five seedlings per pot. The experiment was arranged in a split-plot design on a bench top in a glass-house and irrigated to field capacity twice a week. Plants in half of the pots in each treatment were harvested two times during the experiment with a four weeks interval after the transplantation. Data were recorded for root/shoot length, number of leaves, leaf area per plant and fresh and dry weight. The plant material was analyzed bio-chemically (The method of sample preparation is given in chapter 4 of this thesis) for protein (Peterson, 1984), sugar (Bergmeyer, 1978) and chlorophyll (Knudson *et al*., 1977). Data obtained were statistically analyzed with a one-way ANOVA according to Little and Hills (1978) using MSTATC version 1.42.

Table 3. Physico-chemical characteristics of non-saline and saline-sodic experimental soils. Averages calculated of 5 replications. SP = Saturation Percentage, SAR = Sodium Absorption Ratio, ESP = Exchangeable Sodium Percentage.

	Non-saline	**Saline-sodic**
SP %	19.3	24.9
pH	7.6	9.5
EC_e dS m^{-1}	1.1	7.9
CO_3^{2-} meq l^{-1}	Nil	4.0
HCO_3^- meq l^{-1}	2.5	12.0
Cl^- meq l^{-1}	7.5	280.0
Ca^{2+} meq l^{-1}	10.5	2.0
Mg^{2+} meq l^{-1}	3.0	1.2
Na^+ meq l^{-1}	4.0	93.0
K^+ meq l^{-1}	2.0	0.8
SAR	1.5	73.8
ESP	1.01	51.7
Soil Texture	Clay Loam	Clay Loam

Results

Soil characteristics

The pH of the non-saline soil varied at different depths (8.0-8.4 at 0-15 cm and 8.5-8.7 at 15-30 cm). and increased after the harvest of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. The pH of the saline-sodic soil was greater compared to the non-saline soil. In the saline-sodic soil the pH decreased after harvest (Table 4). The EC_e of the non-saline soil decreased after harvest at 0-15 cm depth. An increase in the EC_e of the non-saline soil was observed at 15-30 cm soil depth. The EC_e of the saline-sodic soil decreased at both soil depths (0-15 and 15-30 cm) after harvesting of the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral crop. However, the difference in the EC_e of saline-sodic soil before and after the harvest of crop was larger than in the non-saline soil (Table 4).

Table 4. Analysis of the soil saturation extract before sowing and after the harvest of fodderbeet at Ratta Kulachi, D. I. Khan of a composite soil sample made up of 3 sub-samples to cover soil patchiness.

Sample taken	**Depth cm**	**pH**		**EC_e dS m^{-1}**	
		Non-saline	**Saline-sodic**	**Non-saline**	**Saline-sodic**
Before sowing	0-15	8.0	8.2	2.7	11.4
	15-30	8.4	8.3	1.1	6.7
After harvest	0-15	8.5	7.6	0.9	7.4
	15-30	8.7	8.0	1.4	5.6

Plant growth and chemical analysis

The fresh weights of the beets increased significantly with increasing soil salinity in the *Beta vulgaris* subsp. *vulgaris* cultivars Monored and Majoral while a significant decrease in the fresh weight of the beets was recorded in *Beta vulgaris* subsp. *vulgaris* cvs. Monoval and Polygroeningia. The fresh weight of leaves increased under saline conditions in all the cultivars (Table 5). The increase in the fresh weight of fodderbeet leaf of *Beta vulgaris* subsp. *vulgaris* cultivars Monored and Majoral was almost three-fold (Table 5).

Table 5. Biomass of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars as affected by the presence of salinity in the soil at Arid Zone Research Sub-station, Ratta Kulachi, D. I. Khan. Averages calculated of 5 replications. One-way ANOVA was computed for significance of soil salinity for each cultivar separately. ns = non-significant, * = P<0.05.

	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia**
	Average fresh weight beet (g)			
Non-saline	867	1142	933	1408
Saline-sodic	525	1492	1317	1367
Salinity	*	*	*	*
	Average fresh weight leaf (g)			
Non-saline	317	400	375	417
Saline-sodic	400	1275	1392	1042
Salinity	*	*	*	*

Chlorophyll ‘a’ and ‘b’ concentrations of fodderbeet cultivars grown under non-saline soil was lower in all the cultivars compared to saline-sodic soil. The ratio between the chlorophyll ‘a’ and ‘b’ concentrations of all the cultivars was also higher on saline-sodic soils were compared to the non-saline soils, except that of *Beta vulgaris* subsp. *vulgaris* cv. Monored (Table 6).

Table 6. Salinity-induced changes in the chlorophyll concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars grown under salt stress conditions at AZRSS, Ratta Kulachi, D. I. Khan. Averages of 5 replications. One-way ANOVA was computed for significance of soil salinity for each cultivar separately. Ns = non-significant, * = P<0.05.

	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris cv.* Polygroeningia**
	Chlorophyll a concentrations (µg chlorophyll/g fresh weight)			
Non-saline	390	560	490	270
Saline-sodic	650	620	970	1220
Salinity	*	*	*	*
	Chlorophyll b concentrations (µg chlorophyll/g fresh weight)			
Non-saline	290	270	600	210
Saline-sodic	420	560	670	500
Salinity	*	*	*	*
	Chlorophyll a/ b			
Non-saline	1.34	2.07	0.82	1.29
Saline-sodic	1.55	1.11	1.45	2.44
Salinity	*	*	*	*

The sugar concentrations in the beets of all the fodderbeet cultivars were low when grown on non-saline soil compared to saline-sodic soil conditions. An increased concentration of sugar was recorded in all the fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars under saline-sodic soil conditions.

Table 7: Salinity-induced changes in the sugar concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars grown under salt stress conditions at AZRSS, Ratta Kulachi, D. I. Khan. Averages calculated of 5 replications. One-way ANOVA was computed of soil salinity for significance of each cultivar separately. ns = non-significant, * = P<0.05.

	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia**
	Sugar concentrations beets (µg g^{-1} fresh weight)			
Non-saline	250 ±50	280 ±50	310 ±60	310 ±60
Saline-sodic	430 ±90	590 ±80	480 ±60	500 ±70
Salinity	*	*	*	*
	Sugar concentrations leaves (µg g^{-1} fresh weight)			
Non-saline	50 ±3	20 ±2	50 ±1	30 ±4
Saline-sodic	40 ±3	20 ±2	30 ±2	30 ±6
Salinity	ns	ns	*	ns

However, the lowest sugar concentration was observed in *Beta vulgaris* subsp. *vulgaris* cv. Monoval under non-saline as well as saline-sodic conditions. The sugar concentrations of fodderbeet leaves were significantly lower than that of the beets. The sugar concentrations of the leaves in all the fodderbeet cultivars did not change under saline-sodic soil conditions as compared to non-saline soil conditions (Table 7). However, there was a decrease observed in the sugar concentration of *Beta vulgaris* subsp. *vulgaris* cv. Majoral under saline-sodic soil conditions.

The protein concentrations of fodderbeet cultivars (beet) were lower under non-saline soil conditions than under saline-sodic conditions. However, sugar concentrations decreased in the beet of *Beta vulgaris* subsp. *vulgaris* cv. Monored under saline-sodic soil conditions. The protein concentrations of fodderbeet leaves in all the cultivars increased under saline soil conditions when compared to those of plants grown under non-saline soil (Table 8).

Table 8: Salinity-induced changes in the protein concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral grown under salt stress conditions at AZRSS, Ratta Kulachi, D. I. Khan. Averages of 5 replications. One way ANOVA was computed of soil salinity for significance of each cultivar separately. ns = non-significant, * = P<0.05.

	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia**
	Protein concentrations beets (mg g^{-1} fresh weight)			
Non-saline	1.1 ±0.2	2.0 ±0.4	1.9 ±0.4	1.8 ±0.4
Saline-sodic	1.7 ±0.3	1.9 ±0.3	2.4 ±0.3	2.0 ±0.3
Salinity	*	*	*	*
	Protein concentrations leaves (mg g^{-1} fresh weight)			
Non-saline	1.8 ±0.4	1.8 ±0.3	1.7 ±0.2	2.0 ±0.1
Saline-sodic	2.6 ±0.4	1.8 ±0.3	2.4 ±0.4	2.9 ±0.3
Salinity	*	ns	*	*

The proline concentrations strongly increased in all the fodderbeet cultivars grown under saline-sodic soil, particularly in the beet tissue of fodderbeet cultivars (Table 9).

Table 9. Salinity-induced changes in the proline concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral grown under salt stress conditions at AZRSS, Ratta Kulachi, D. I. Khan. Averages calculated of 5 replications. One-way ANOVA was computed of soil salinity for significance of each cultivar separately. ns = non-significant, * = P<0.05, ** = P<0.01, *** = P<0.001

	***Beta vulgaris* subsp. *vulgaris* cv. Monoval**	***Beta vulgaris* subsp. *vulgaris* cv. Monored**	***Beta vulgaris* subsp. *vulgaris* cv. Majoral**	***Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia**
	Proline concentrations beets (µg g^{-1} fresh weight)			
Non-saline	700 ±60	750 ±90	470 ±20	950 ±90
Saline-sodic	2380 ±240	2260 ±170	1800 ±90	1890 ±120
Salinity	***	**	***	**
	Proline concentrations leaves (µg g^{-1} fresh weight)			
Non-saline	780 ±30	210 ±10	530 ±20	490 ±60
Saline-sodic	1750 ±240	1330 ±150	1210 ±180	1080 ±110
Salinity	**	***	**	**

In experiment II, the biomass production of fodderbeet grown under saline soil conditions was higher than those of barley and oat. The average number of leaves produced in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was significantly higher than barley and oat leaves. The leaf area per fodderbeet plant measured was two-fold compared to the conventional fodder crops (barley and oat). The leaves of fodderbeet were broader, more outwardly spread and fully turgid, while those of barley and oat were significantly less broad and hanging. The chlorophyll concentrations of fodderbeet leaves were comparatively higher than that of barley and of oat.

The colour of the fodderbeet leaf was comparatively darker. The fresh weight of the whole plant was three-fold that of the conventional crops (barley and oat) (Table 10).

Shoot growth of the fodderbeet increased by the addition of FYM to non-saline soil. Shoot growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral also increased under non-saline soil conditions, but addition of FYM to saline-sodic soil did not improve the shoot growth with the age of plant (Table 11). The root growth of the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral increased under FYM amended non-saline soil however, the root growth decreased under the saline-sodic soil. Addition of FYM to saline-sodic soil decreased the root growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral at harvest I, however, the root length increased significantly at harvest II (Table 11).

Table 10. Growth comparison of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral), barley and oat under field conditions. Figures with different letters in each row are significantly different at P<0.01. Lettering is ranked in descending order.

Growth parameters	**Unit**	**Crops**		
		Fodderbeet	**Barley**	**Oat**
No. of leaves plant^{-1}	-	13.3 a	5.3 b	4.0 b
Leaf area plant^{-1}	cm^2	546.5 a	229.2 b	245.4 b
Chlorophyll ‘a’ and ‘b’	mg/g fresh weight	1.4 a	0.7 b	0.6 b
Fresh weight	kg plot^{-1} (6 x 4 m^2)	15.7 a	5.0 b	4.2 b

Table 11. Effect of FYM application on growth parameters of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plant under non-saline and saline-sodic soil conditions after 8 weeks (harvest I) and 16 weeks (harvest II). Two-way ANOVA computed for significance of salinity, FYM and their interaction (salinity * FYM) for each parameter and each harvest separately. Averages calculated of 10 replicates. ns = non-significant, * = P<0.05.

	Non-saline soil		**Saline-sodic soil**	
	- FYM	**+ FYM**	**- FYM**	**+ FYM**
		Shoot height (cm)		
Harvest I	11.3	40.4	27.6	39.8
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Harvest II	11.3	41.5	28.6	43.3
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
		Root length (cm)		
Harvest I	7.3	10.3	6.3	5.6
Salinity	ns	*	ns	*
FYM	*	*	ns	ns
Salinity*FYM	ns	*	*	ns
Harvest II	8.9	11.1	7.4	10.6
Salinity	ns	ns	ns	ns
FYM	*	*	*	*
Salinity*FYM	ns	*	*	ns
		Number of leaves		
Harvest I	8.1	12.8	9.3	12.5
Salinity	ns	ns	ns	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Harvest II	8.9	15.4	9.4	13.7
Salinity	ns	ns	ns	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*

The number of leaves of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral increased under FYM amended non-saline soil. Addition of FYM to saline-sodic soil did not change the number of leaves compared to non-saline soil (Table 11).

The fresh weight of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plants significantly increased with application of FYM to the non-saline soil. Plants grown in the saline-sodic soil without any amendment produced 11 times more fresh weight when compared to the non-saline soil. Fresh weight of plants grown in the FYM-amended saline-sodic soil was similar to the FYM-treated non-saline soil. The leaf area per fodderbeet plant increased under non-saline soil amended with FYM. Addition of FYM to saline soil also significantly affected the leaf area per plant (Table 12).

Analyses for protein, sugars and proline concentrations were carried out at the fist harvest only (eight weeks). The protein concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral were lower under saline-sodic soil conditions. Addition of FYM to non-saline soil increased the protein concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Protein concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral did not change under FYM-amended saline-sodic soil conditions (Table 13). The chlorophyll ‘a’ and chlorophyll ‘b’ concentrations increased in the presence of FYM in the non-saline soil. The FYM added to saline-sodic soil decreased the chlorophyll ‘a’ concentrations of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. However chlorophyll ‘b’ concentrations was higher on FYM-amended saline-sodic soil (Table 13).

Table 12. Effect of FYM application on the biomass of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plant under non-saline and saline-sodic soil conditions after 8 weeks (harvest I) and 16 weeks (harvest II). Two-way ANOVA computed for significance of salinity, FYM and their interaction (salinity * FYM) for each parameter and each harvest separately. Averages calculated of 10 replicates. ns = non-significant, * = $P < 0.05$.

	Non-saline Soil		**Saline-sodic Soil**	
	- FYM	**+ FYM**	**- FYM**	**+ FYM**
Fresh weight plant^{-1} (g)				
Harvest I	2.6	65.4	22.5	64.0
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Harvest II	2.7	122.6	25.6	83.9
Salinity	*	*	*	*
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Dry weight plant^{-1} (g)				
Harvest I	0.2	2.1	1.2	2.2
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Harvest II	0.2	8.2	2.9	6.5
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Average leaf area plant^{-1} (cm^2)				
Harvest I	8.8	99.7	52.7	112.6
Salinity	*	ns	*	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Harvest II	9.6	137.8	52.2	112.2
Salinity	*	*	*	*
FYM	*	*	*	*
Salinity*FYM	*	*	*	*

Table 13. Effect of FYM application on the protein (µg ml^{-1}) and chlorophyll 'a' and chlorophyll 'b' (mg g^{-1} fresh weight) concentrations of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) plant under non-saline and saline-sodic soil conditions after 8 weeks (harvest I). Two-way ANOVA computed for significance of salinity, FYM and their interaction salinity * FYM for each parameter and each harvest separately. Averages of ten replicates. ns = non significant, * = P< 0.05.

	Non-saline soil		**Saline-sodic soil**	
	- FYM	**+ FYM**	**- FYM**	**+ FYM**
Protein concentrations (µg ml^{-1})				
Harvest I	776.3	1446	692.5	1523
Salinity	ns	ns	ns	ns
FYM	*	*	*	*
Salinity*FYM	*	*	*	*
Chlorophyll 'a' concentrations (µg g^{-1} Fresh weight)				
Harvest I	350	450	410	400
Salinity	*	*	*	*
FYM	*	*	ns	ns
Salinity*FYM	*	ns	ns	*
Chlorophyll 'b' concentrations (µg g^{-1} Fresh weight)				
Harvest I	90	120	110	170
Salinity	ns	*	ns	*
FYM	*	*	*	*
Salinity*FYM	*	ns	ns	*

Discussion

Effect of plant growth on soil characteristics

The pH and EC_e of the saline-sodic soil in the upper 0-15 cm depth before sowing of the crops was high compared to non-saline soil. The pH and EC_e

of the saline-sodic soil were decreased significantly in the upper 0-15 cm of soil at the time of harvest (Table 4). The reduction in the pH and EC_e of soil may be a sign of improvement in the physico-chemical characteristics of the soil. Addition of farm yard manure to the saline soil may produce humic acids by microbial action and decomposition of organic material (Tisdale, *et al*., 1985). The saline and saline-sodic soils are mostly calcareous in nature in Pakistan. The release of protons during the exchange of Na^+ with Ca^{2+} on the soil complex may be one of the reasons of a lower pH of soil after harvesting. Therefore, regular cropping of fodderbeet may support further improvement of the soils. The reduction of the EC_e in the soil surface layer may indicate uptake of salt by the plants. The dissolution of salts present with the soil particles due to a lower soil pH and leaching of these salts to deeper layers may have reduced the EC_e of the soil in the upper layers of soil. The higher pH and EC_e in the deeper layer (15-30 cm) is indicative of leaching of the salts to deeper layers due to a change in the reaction of soil in the presence of growing plants. In the solution culture experiments (chapter 3 and 4) the pH (5.5) and EC of the growth medium was maintained regularly. In the pot-culture (chapter 4), the movement of salts was also restricted within the soil depth in the pot.

Plant biomass accumulation under saline-sodic soil conditions

The fresh weight of beets and leaves increased in *Beta vulgaris* subsp. *vulgaris* cvs. Monored and Majoral with increasing salinity (Table 5). The *Beta vulgaris* subsp. *vulgaris* cv. Majoral accumulated a higher biomass than the other cultivars. Magat and Goh (1988) recommended NaCl as fertilizer for production of fodderbeet. In contrast, application of various doses of K (0-60 mg 100 g^{-1} soil) did not significantly improve the leaf and root growth of sugarbeet on an alluvial soil (Beringer *et al*., 1986), which indicates a limited K^+ requirement of sugarbeet. Soil texture plays an important role in the growth of plant under saline soil conditions. Nutrients in clay soil helped in increasing the accumulation of biomass in two species of *Salicornia* under non-flooded and flooded conditions while growth of plant was retarded under saline conditions. The change in dry matter upon flooding became non-significant under non-saline conditions (Rozema *et al*., 1986; 1987).

Concentration of organic molecules

The chlorophyll ‘a’ and ‘b’ concentrations were increased significantly under saline conditions (Table 6). Increase in chlorophyll ‘a’ concentrations under saline conditions was significant in *Beta vulgaris* subsp. *vulgaris* cvs. Monoval, Polygroeningia and Majoral. The chlorophyll a/b ratio revealed that chlorophyll ‘b’ concentrations increased under saline conditions in all the cultivars, except in *Beta vulgaris* subsp. *vulgaris* cv. Monored. This may be a reason for the darker colour of the leaves under saline conditions. Papp *et al.* (1983) reported a decrease in the chlorophyll concentrations under saline conditions in sugarbeet. The decrease in chlorophyll concentrations has been related to the increase in self-shading of mesophyll cells due to leaf thickness of sugarbeet under saline conditions. Total sugar in beet was comparatively higher under saline conditions (Table 7). While in leaves, the sugar concentrations were comparatively low, indicating a better downward movement and storage of sugar in the beet under saline conditions (Table 7). The protein concentrations of leaves were comparatively high under saline conditions. Similarly, the proline concentrations of beets and leaves were significantly increased by salinity (Table 8). A salinity-induced reduction in leaf weight was shown during the earlier growth period in sugarbeet (Plaut and Heuer, 1985). A significant increase in the root fresh weight compared to the leaves was noted in the present study (Table 5) which may reflect a high salt tolerance and adjustment of fodderbeet to salinity and possibly efficient transportation of photosynthates from the leaf to the root. These results could be supported by chlorophyll ‘a’ and ‘b’ concentrations of the plants under non-saline and saline-sodic soil conditions (Table 6). A significant increase in chlorophyll ‘a’ and ‘b’ concentrations and an increased photosynthetic rate may have added considerably to the fresh weight of the plants. Products of photosynthesis may also be important for osmoregulation and may help in the evaluation of the degree of salt tolerance of the plants. A significant reduction in pH and EC_e of the saline soil may have improved the soil condition and increased ion concentrations and hence improved the physiological functions of the plants, i.e. photosynthesis. Increases in the leaf protein and proline concentrations may also relate to the improved physiological processes involved in salt tolerance of fodderbeet (Table 8 and 9). An increase in the free proline accumulation in petunias under drought stress was reported by Bartels and Sankar (2005). Proline is synthesized both under drought and salt stress. Petunia plants produced 8 times more proline content compared to that of control plants under drought conditions (Bartels and Sankar, 2005). Rabbani *et al*. (2003) also reported that many plants accumulate compatible osmolytes such as proline, betaine or sugars under osmotic stress. Several physiological studies suggested that under stress conditions sugars also accumulate to varying degree in different plant species (Taji *et al*., 2002). Of the total osmolytes sugars contribute up to 50 % of the total osmotic potential in glycophytes depending on the saline conditions (Cram, 1976). Under saline conditions proteins are accumulated in plant as salt stress proteins (Rabbani *et al*., 2003).

The fodderbeet plant seems to be halophytic and may be even halophyllic since uptake of Na^+ and Cl^- ions promoted its growth. Sodium chloride is applied as fertilizer in some soils of New Zealand for increased growth of fodderbeet (Magat and Goh, 1988). The field having non-saline soil used for the experiment in this chapter was a reclaimed soil. Addition of reclamant (gypsum) may have solubilized the excessive sodium ions alongwith nutrients. These nutrients may have leached down with the irrigation water to deeper layers of the soil, leaving the surface soil deficient in nutriens.

The chlorophyll ‘a’ and ‘b’ concentrations were significantly higher under saline conditions (Table 6). The increase in chlorophyll ‘a’ concentrations under saline conditions was highly significant in *Beta vulgaris* subsp. *vulgaris* cvs. Monoval, Polygroeningia and Majoral. Chlorophyll a/b ratio revealed that chlorophyll b increased under saline conditions in all the cultivars except in *Beta vulgaris* subsp. *vulgaris* cv. Monored. The higher protein concentrations of the leaves (Table 8) and the proline concentrations of beets and leaves were significantly increased by salinity (Table 9), which may indicate an improvement in physiological processes related to salt tolerance of fodderbeet.

Comparison of growth of conventional and non-conventional fodder crops

The fresh weight produced by fodderbeet under field conditions was significantly ($P<0.01$) greater than that of barley and oat. It was directly influenced by a significantly higher number of leaves per plant and a higher leaf area per plant in fodderbeet (Table 10). Lenssen *et al*. (1995) reported a reduction in the leaf area and the dry weight of roots of *Aster tripolium* in the presence of high salinity. However, the dry weight of stems was not affected. In fodderbeet, the difference in dry weights of roots and shoots were more prominent.

The significantly higher chlorophyll concentrations of fodderbeet compared to oat and barley reflect an adaptation under saline-sodic conditions in that crop (Table 10). Barley and oat crops have lower salinity tolerance compared to fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral, therefore their plant growth was reduced on saline-sodic soil. Growing fodderbeet on a large scale may not help in reducing the shortage of fodder for the cattle in the area but it may also reduce soil salinity by decreasing the pH and EC of the salt-affected land. The improvement of saline-sodic soils may encourage farmers of the area to adopt fodderbeet as a fodder crop on saline-sodic fields to improve their lands. On the other hand barley and oat are also cultivated for grain production for human food in that area. At the same time they may cultivate barley and oat on non-saline soil. In this way, the income of the farmers of the area may be enhanced.

Response of fodderbeet growth under FYM-amended saline-sodic soil

In a pot experiment using saline soil and FYM, different growth characteristics of fodderbeet displayed a variable response to soil condition (Table 3). The plants showed low shoot height in the normal soil, but the plant height increased with the application of farm yard manure. Improvement in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral shoot height was also observed with the application of FYM in the saline-sodic soil. Interactions of FYM with salinity were very significant and a depressing effect on the shoot height of fodderbeet was observed in the presence of FYM in saline-sodic soil compared to non-saline soil amended with FYM. At the time of the first harvest (eight weeks after transplantation), plants achieved the maximum height, which stayed constant until the time of second harvest (Table 11).

Fodderbeet is known to be sensitive to salt stress at germination, but it is tolerant to increased salinity during vegetative growth (Rozema *et al*., 1993). The growth of fodderbeet was increased in saline-sodic soil compared to the non-saline soil. Addition of farm yard manure improved the soil characteristics by increasing the availability of ions to the plants (More and Ghonsikar, 1988). Farm yard manure also plays an ameliorative role by decreasing the desorption capacity of soil followed by an increase in certain soluble ions (Sharif *et al*., 1974).

The root length of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral increased with the application of FYM to non-saline soil. A high concentration of salt tends to slow down or stop root elaongation (Kramer, 1983) and causes reduction in root production (Garg and Gupta, 1997). Addition of FYM to saline-sodic soil did not affect the root length at harvest I. A significant effect was observed for the number of leaves per plant and the plant height at both harvests. The height of fodderbeet increased at 8 weeks of growth, which helped in the increase of biomass. The photosynthates were mostly utilized in the energy supply for the cell division, growth and development of the shoots. The photosynthetic material may not be translocated to the roots during this period, which resulted in a slow root growth. The root of fodderbeet may serve as a storage organ, i.e. a sink for photosynthates. The development of the roots after the second harvest (16 weeks) may relate to translocation of photosynthates to this sink.

Relatively low fresh and dry weights and average leaf areas per plant were produced in non-saline soils without FYM application. A 6-8 fold increase in non-saline soils with FYM amendment was recorded in these parameters. In the absence of FYM application, saline-sodic soils produced significantly higher fresh weight of plants in both harvests. Application of FYM to saline-sodic soils produced lower fresh weights of plants at harvest II when compared to non-saline soils (Table 12). An increase in the average leaf area per plant was observed in the first harvest but during the second harvest, the leaf area per plant decreased on non-saline and saline-sodic soils, respectively. It may be noted that an increase of fresh and dry weights occurred after the second harvest in all the treatments, except in non-saline soils without FYM application. A significant increase in biomass production could be related to the number of leaves per plant and to the average leaf area per plant. Both the above-mentioned growth characteristics may relate to maximum light energy interceptions by the plants (Niazi *et al*., 2000). Garg and Gupta (1997) reported that salinity caused reduction in leaf area as well as in rate of photosynthesis which combined result in reduced crop growth and yield.

The protein concentrations of fodderbeet roots were lowest under non-saline soil (Table 13). An increase of 29% in protein concentrations was noted under saline-sodic soil conditions. Addition of FYM to normal soil increased the protein concentrations up to 37%; however, addition of FYM to saline-sodic soil also improved the protein concentration (Table 13). The beneficial effects of FYM application on protein concentrations of fodderbeet were observed in normal soils and saline-sodic soil. Plaut and Heuer (1985) have reported a significant increase in protein content of sugarbeet under saline conditions. The increase in protein content has been related to the supply of nutrients (Firgany *et al.*, 1981). FYM applied in the present study may relate to the supply and release of nitrogen along with the micronutrient. This may stimulate some enzymes, which may be involved in the increase of protein synthesis. Chlorophyll 'a' and 'b' concentrations remained unaffected. Chlorophyll ‘a’ concentrations were 3 to 4 times higher than those of chlorophyll 'b' in all treatments. However, no significant effects of soil type and FYM application were observed on either chlorophyll 'a' or 'b' concentrations of fodderbeet leaves (Papp, *et al*., 1983). The increase in the average leaf area per plant may have decreased the chlorophyll concentration by dilution, thus smaller differences in the values were recorded. In the present study, the FYM was added to the saline-sodic soil expecting an improvement in the growth of fodderbeet plant by providing nutrients to the plant under saline conditions. But the present results showed that although the interaction of FYM was significant in most of the parameters studied, however the interaction of FYM and salinity is negative and growth of the plant was depressed under saline-sodic soil amended with FYM. On the other hand addition of FYM to the non-saline soil helped in the availability of nutrients for better growth of plant.

The fodderbeet plant grows well under saline (non-sodic) soil conditions during the vegetative growth stage (Niazi, *et al*., 1999a). Saline-sodic soil conditions may inhibit the growth by reducing the availability of ions to the plant. Addition of FYM could help plant growth by affecting the pH and EC_e of saline-sodic soils (Tisdale *et al*., 1985). The FYM also activates the activity of nitrifying micro-organisms. In addition, the cation exchange loss of inorganic nitrogen (NH_4^+) is improved (Gasser, 1964). FYM in turn may help the release of essential ions to the soil solution and make these ions available to the plants resulting in increased growth of the plants.

Conclusion

The results obtained from the growth of fodderbeet in the saline-sodic soils at Dera Ismail Khan, (N.W.F.P) were found favourable for the cultivation of fodderbeet. The growth of *Beta vulgaris* subsp. *vulgaris* cv. Majoral was increased compared to other fodderbeet cultivars (Monored, Monoval and Polygroeningia) (Experiment I). The increased biomass of *Beta vulgaris* subsp. *vulgaris* cv. Majoral compared to conventional fodder crops (barley and oat) suggests that *Beta vulgaris* subsp. *vulgaris* cv. Majoral may be successfully grown on the salt-affected soils of D. I. Khan. The crop could solve to some extent the problem associated with utilization of saline soils. The shortage of green fodder during the winter in this area may be overcome to some extent by cultivation of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. The agro-ecological zones in Punjab and upper Sind provinces of Pakistan have similar types of environment and a vast area represents saline and saline-sodic arable lands. This area may also be tested for the cultivation of fodderbeet. A number of experiments have already been conducted in the experimental field at the Saline Agriculture Sub-station at Sadhuke, near Lahore, Punjab province of Pakistan and at the Sugar crops Research Institute, Mardan.

However, saline-sodic soil conditions may inhibit the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral non-significantly. Addition of FYM as an amendment to the non-saline soils may improve the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plant. Addition of FYM to saline-sodic soils may enhance the fodderbeet plant growth. A significant increase in the average leaf area per plant and the mass of the roots in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral), may play an important role to increase the biomass production. The winter cropping season in Pakistan coincides with the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral cultivation. Therefore, introduction of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral in problem soils can improve the fodder availability during the winter in Pakistan.

CHAPTER 6

Chapter 6

General Discussion

<u>Global salinization</u>

Around 6% of the global land area suffers from salinization due to natural causes or irrigation, posing a major strain on agricultural production. It is estimated that irrigation related salinization leads to the abandonment of 10^7 hectares of agricultural land annually (Frans, *et al*., 2001). In Pakistan, field crops are mainly irrigated by canals, however, about one third of the total cultivated land is rainfed, which is variable and predictable. Of the total 6.2 million ha saline/sodic soil, cultivated portion is 45% and uncultivated portion is 55% (GOP, 2003a). Soils having mainly surface or patchy salinity/sodicity include 0.6 million ha and 1.3 million ha of moderately saline soils. Moreover, 40% of the total ground water is useable, out of remaining 60%, 17% is marginally saline or saline-sodic and 43% is hazardous (Kahlown and Khan, 2002). Excess of salts in the soil may be harmful for the growth of plants. However, some halophytes successfully grow in these environments. All the halophytes may not be useful and tasteful for human beings. Efforts are needed to explore those halophytes that can be used as human food indirectly, by utilization through animals. According to Burman *et al*., (2003) there are detrimental effects due to salinity and drought on plant water status, net photosynthesis, leaf metabolite levels and nitrate reductase activity. Salinity affects plant physiology through changes of water and ionic status in the cells (Hasegawa *et al*., 2000).

<u>Halophytes and biosaline agriculture</u>

The use of some halophytes for rehabilitation and reclamation of salt-affected lands has proven to be feasible. About one quarter of the world halophytes are Chenopodiaceae (Aronson, 1989). Fodderbeet (*Beta vulgaris* subsp. *vulgaris*) is grown in coastal areas of the Netherlands, the United Kingdom, Germany and other parts of Europe (Magat anf Goh, 1988; Rozema, 1991). Therefore non-conventional salt-tolerant fodder plants may be grown to make up the fodder requirement of cattle and the objective of utilization of salt-affected soil may be achieved as well (Rozema, 1991). Fodderbeet is a cultivar domesticated from seabeet that is found along the coastal areas of European countries under natural saline conditions. In Pakistan, fodderbeet can be introduced as a non-conventional fodder crop in salt-affected areas, which may help in reducing the acute shortage of fodder for animals during winter. Fodderbeet is reported to be a relatively salt-tolerant crop at the vegetative stage (Rozema *et al*., 1992). Reports on successful cropping of fodderbeet are also available from Russia and New Zealand (Popovic and Stikie, 1986; Magat and Goh, 1988). Application of NaCl (295-1180 kg ha^{-1}) is required for a significant increase in the tops and the root yield of fodderbeet (Goh and Magat, 1989). The halophilous nature of fodderbeet was reported by Magat and Goh (1988). A comparatively higher concentration of chlorophyll in fodderbeet has been reported by Niazi *et al.* 1999; Maas, *et al*., 1986; Maas and Nieman, 1978) under saline soil conditions.

<u>Fodderbeet as a crop for biosaline agriculture</u>

Fodderbeet cultivation in saline flood plain soils in Pakistan implies germination of seed and seedling establishment in saline, sometimes sodic soils. Salt tolerance of plants varies greatly during different phases of growth and development. Feeding of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) to dairy cows significantly improved fat and protein content of milk and the yield (Roberts, 1987). The salinity of salt-affected arable land in Pakistan may be up to 20 dS m^{-1} (~ 200 mM NaCl). The fodderbeet cultivar *Beta vulgaris* subsp. *vulgaris* cv. Majoral has been reported to be a salt tolerant plant up to 150 mM NaCl during the vegetative growth period (Niazi *et al.* 1995). A comparative study of *Beta vulgaris* subsp. *maritima* and domesticated *Beta vulgaris* subsp. *vulgaris* cv. Majoral grown under saline soil conditions could give an idea about its successful cultivation in Pakistan as well. Hence, a number of studies on fodderbeet were planned under artificially salinized conditions to evaluate a successful fodder crop especially for small farmers of salt-affected lands in developing countries.

Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is known to be sensitive during germination but tolerant to increased salinity during later growth stages (Bernstein and Hayward, 1958). There is considerable variation in the response of seed germination and crop growth to increased salinity, but generally seed germination is decreased with increasing salinity. At the optimal temperature regime of 20-30 ^{o}C, seeds of fodderbeets showed 93 % germination at 0 mM NaCl. The germination rate decreased to 18 % with increasing salt concentration to 500 mM NaCl (Gulzar and Khan, 2002). Temperature shifts may affect a number of processes determining germinability of seeds including membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994). After germination during the wet and moist (and non-saline) winter seabeet *Beta vulgaris* subsp. *maritima* seedlings may have successfully established (Rozema, 1975). In our study, temperature was kept constant and no diurnal change occurred. The seed germination rate of fodderbeet was high (80 – 90 %) and varied slightly with temperature (20^{o}, 25^{o} and 30 ^{o}C) (Chapter 2, Table, 1 - 3). Fodderbeet seed germination at 25 ^{o}C was somewhat higher than at 20^{o} and 30 ^{o}C. This high percentage of germination at these temperatures agrees partly with the moderate (Atlantic Coast) to warm climate (Mediterranean coast of Europe and Africa). At the salinity level of 20 dS m^{-1} (200 mM NaCl) roughly equaling 40% of the salinity of seawater (500 mM NaCl), the response of fodderbeet seed germination was about 20%. Fodderbeet seed germination of different cultivars was high (94 – 75 %) under low-saline (4 dS m^{-1}) conditions at temperatures from 20^o – 30 ^oC (Chapter 2, Table, 1-3). Based on the seed germination response to salinity and temperature variation, fodderbeet could be grown as a winter crop by direct sowing in Pakistan.

Salt tolerance of crops and growth analysis

It was found that the salt tolerance assessed as the mean relative growth rate at 400 mM NaCl and at 0 mM NaCl of the halophyte coastal seabeet *Beta vulgaris* subsp. *maritima* is about 10% higher than in the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral (Chapter 3). The analysis of growth parameters revealed that the Leaf Area Ratio and Specific Leaf Area are most affected by increasing salinity in the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral (Chapter 3, Table 1). Under saline conditions, the growth of some halophytes is generally stimulated and accompanied by accumulation of Na and Cl ions particularly in the leaves (Flowers *et al*., 1977; Watkins *et al*., 1988). Accumulation of these ions in the leaves may provide opportunities to some halophytes to survive under fluctuating NaCl concentrations in their environment. The concentrations of K^+, Ca^{2+} and Mg^{2+} were depressed by salinization at variable NaCl levels (Chapter 3, Table 4). These levels probably constitute basic and minimal concentrations of these ions in the tissues for satisfactory growth to occur. The increased growth rate of the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) at 150 mM NaCl in the garden soil at harvest II (Chapter 4, Table 2) implies that the crop may be successfully cultivated in salt-affected arable land such as occurring in Pakistan (Rozema, *et al*., 1990). Similar results were presented by Niazi *et al*. (2005), Ashraf *et al*. (2003), Niazi *et al*., (2000) and Vivanco *et al*. (2002).

Different plant species have shown salt sensitivity at various growth stages (Boardman, 1977; Niazi, *et al*., 1999 &1999a). Chenopodiaceae and Gramineae (Poaceae) show different behaviour for biosynthesis of glycinebetaine, which is a quaternary ammonium compound accumulating in the leaves of a wide range of species (Boardman, 1977; Haeder and Mengel, 1972; Papp, *et al*., 1983). Accumulation of glycinebetaine increases considerably under saline conditions in species belonging to the Chenopodiaceae or Gramineae (Poaceae) (Haeder and Mengel, 1972; Papp, *et al*., 1983). The K^+ levels in plant tissues interact with some proteins and regulate the functions of some molecules to regulate assemblage of targeting ion channels (Sinnige, *et al*., 2005; Bunney, *et al.*, 2002). Therefore, measurement of ion concentrations like Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+} and levels of some osmolytes (sugars, glycinebetaine and protein) of plant parts (roots and leaves) was included in addition to biomass production of fodderbeet and seabeet.

A comparison of RGR values of fodderbeet cultivars and seabeet revealed that RGR values in fodderbeet were higher under salinity (150 mM NaCl) than those in seabeet (*Beta vulgaris* subsp. *maritima*) (Chapter 4, Table 2). The values of RGR were within the range of herbaceous plants mentioned by Poorter (1989). The RGR of fodderbeet and seabeet decreased significantly during the period of six weeks of experiment I. The NAR increased for *Beta vulgaris* subsp. *vulgaris* cultivars Majoral and Monored, in the presence of 150 mM NaCl. The NAR significantly increased over six weeks compared to the first harvest. The leaf area per plant also increased significantly during this period (Chapter 4, Table 4). The rate of photosynthesis was increased with increased salinity in *Beta vulgaris* subsp.

maritima. It was related to a significant increase of the leaf thickness in seabeet (Chapter 4, Table 3). The water potential of the shoot did not change under saline conditions except *Beta vulgaris* subsp. *maritima*. However, the osmotic potential was significantly reduced (more negative) at 150 mM NaCl (Chapter 4, Table 2). A significant increase in leaves and roots dry weight of *Beta vulgaris* subsp. *maritima* was recorded with increased salinity (150 mM NaCl) in the first two weeks, while in the *Beta vulgaris* subsp. *vulgaris* cv. Majoral, a reduction in dry weight of leaves and roots was observed under saline conditions (Chapter 4, Table 4). A lower number of leaves per plant in fodderbeet cultivars compared to seabeet under increasing salinity may relate to reduced dry weight of shoot biomass. *Beta vulgaris* subsp. *maritima* had the highest average number of leaves, but the total leaf area per plant was less compared to the fodderbeet cultivars due to smaller leaf area of the individual leaf. The leaves of *Beta vulgaris* subsp. *maritima* were succulent.

Fodderbeet plants possibly used up most of the energy available for ion pumps involved in compartmentation and secretion and repair of cellular damage caused by the saline conditions (Penning de Vries, 1975; Schwarz and Gale, 1981) to overcome the effect of salinity. Also the hormonal and physical (i.e. turgor potential) factors controlling cell wall loosening and cell expansion (Cleland, 1986) may have adjusted during the growth period of the fodderbeet. The amount of photosynthates translocated to the roots under saline conditions was relatively low during the early growth of the plants, due to which a considerable amount of dry matter accumulated in the shoot system This adaptation may be related to an increase in leaf area with increased salinity (*Beta vulgaris* subsp. *maritima*, +13.8 %) (Chapter 4, Table, 4). The fodderbeet appeared to differ with respect to LAR and LWR.

The difference in LAR and LWR may be related to the significantly negative effect of salinity treatment on SLA in all cultivars except *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia cultivars and seabeet *Beta vulgaris* subsp. *maritima*. SLA was somewhat increased in the presence of salinity in all the cultivars except *Beta vulgaris* subsp. *vulgaris* cv. Polygroeningia and seabeet *Beta vulgaris* subsp. *maritima*).

<u>Fodderbeet and Seabeet</u>

Beta vulgaris subsp. *maritima* is the halophytic ancestor of fodderbeet growing in coastal areas of the Atlantic and Mediterranean, while the fodderbeet cultivars are its domesticated breeds. The wild *Beta vulgaris* subsp. *maritima* has a rosette structure, a greater number of leaves and comparatively narrower leaves than the leaves of fodderbeet cultivars. The leaves of the fodderbeet cultivars are broader and have a significantly larger leaf area. They intercept more light and synthesize more biomass per leaf than seabeet. This may underly the larger RGR of the fodderbeet cultivars compared to seabeet. Similar findings had also been discussed by Rozema *et al*. (1993); Rozema (1990; 1991).

The biomass accumulation of fodderbeet and seabeet shoot was not affected during the first two weeks with increasing salinity (200 mM NaCl). After two weeks, the plants may have adapted to the saline environment and an increase in the biomass of the shoots was significant with increased salinity compared to the control (0 mM NaCl). The dry weight accumulation of the fodderbeet and seabeet during the early vegetative growth was limited but later accumulation of dry matter was significantly higher at 200 mM NaCl than at 0 mM NaCl. This confirmed that the plants had adapted to the saline environment. The fresh weight of the roots increased in the control treatment compared to 200 mM NaCl. Fresh and dry weights of roots of fodderbeet were significantly higher compared to seabeet (Chapter 4, Tables 6 & 7). The difference in the structure of roots of both plants may have resulted in the observed difference in biomass accumulation**.** The fresh and dry weight ratios of the shoots and roots in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) were higher than that of seabeet (*Beta vulgaris* subsp. *maritima*) during the growth period studied (Chapter 4, Table 8). It showed increased biomass accumulation in the fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) roots compared to that of seabeet (*Beta vulgaris* subsp. *maritima*). While comparing two different experiments, the RGR in fodderbeet increased under 150 and 200 mM NaCl within 2 weeks growth period (Chapter 4, table 2 and 18). Comparison of different growth perameters in solution culture and garden soil (Chapter 3, table 5) showed 20% decrease in RGR in seabeet (*Beta vulgaris* subsp. *maritima*) and 30% decrease in fodderbeet (Beta *vulgaris* subsp. *vulgaris* cv. Majoral). In LAR 35% decrease in both fodderbeet and seabeet was noted. A decrease in the SLA of both sub-species was recrded [fodderbeet (36%) and in seabeet (47%)]. However, there was no change observed in NAR, LWR, net photosynthesis and transpiration.

<u>Ion uptake</u>

The presence of a higher concentration of NaCl (200 mM NaCl) in the growth medium increased the uptake of Na^+ by the plant. The Na^+ concentration of the plant tissue increased during growth until the final harvest (Chapter 4, Table 9). Translocation of ions from the roots to the shoots with increased salinity was higher than that at the control treatment. Different plant species differ in the underlying mechanism of Na^+ influx to the plant. The mass flow of soil solution is partitioned at the root surface, the water being taken up and the Na^+ and Cl^- is excluded or pumped out (Tyerman *et al*., 1997; Yadev *et al*., 1996; Yeo, 1998).

Higher Na^+ and Cl^- ion accumulation by the shoots and leaves of *Beta vulgaris* subsp. *maritima* (Chapter 4, Table 9 & 10) confirm the physiological status of many members of the family *Chenopodiaceae* as salt accumulators (Rozema *et al*., 1981). Salt accumulation is considered as one of the indices of salt tolerance (Shannon and Grieve, 1999). In an outdoor sand culture experiment at the U.S. Salinity Lab, an increase in the dry weight of seabeet was reported up to 11 dS m^{-1} (~ 110 mM NaCl) and then the dry weight further reduced at a rate of about 5.7 % per dS m^{-1} (Shannon and Grieve, 1999). The calculated 50 % decrease in the dry weight compared to control for seabeet yield was at an EC of 19.8 dS m^{-1}. Similarly Osawa (1996) reported 50 % decrease in the dry weight of *Beta vulgaris* subsp. *maritima* at 17.5 dS m^{-1} compared to control. In the present study, the dry weight of shoot significantly increased in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) (31%) and seabeet (*Beta vulgaris* subsp. *maritima*) (34%) after 35 days of plant growth (Chapter 4, Table 6) while the dry weight of the roots in fodderbeet increased (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) (17%). In seabeet (*Beta vulgaris* subsp. *maritima*) dry weight of the roots significantly decreased (22%) (Chapter 4, Table 7). The dry weight of root increased in both the sub-species although the salinity level 200 mM NaCl (~ 20 dS m^{-1}) was slightly higher than the salinity level reported by Osawa (1996).

A negative correlation (r^2 = -0.77) was observed between Na shoot/K root ratio in *Beta vulgaris* subsp. *vulgaris* cv. Majoral while *Beta vulgaris* subsp. *maritima* showd a stronger negative correlation value (r^2 = -0.88) than that of fodderbeet (correlation values calculated from the data in chapter 4). An antagonistic effect between Na^+ and K^+ ions during a study on the uptake of ions in tomato under saline soil conditions was found by Niazi and Ahmad (1984). Generally low K uptake by the roots is believed to involve inward-rectifying K channels, allowing K to enter along an electro-chemical gradient (Smart *et al*., 1996; Meidema, *et al.*, 2003). This does not mediate K uptake by the roots (Nakamura *et al*., 1995), and has been proposed as a mechanism of low affinity uptake into guard cells. Sodium is excluded from the shoots by many plants. It is retained in the roots and the lower stem. The latter strategy is only successful at low to moderate external Na^+ concentrations and relies on the selective release of Na^+ into the xylem and its re-sorption from the xylem stream. Resembling interpretations have also been discussed by Maathuis *et al.* (1996), Fu and Luan (1998), Hirch *et al*. (1998), Amtmann and Sanders (1999), and Tyerman and Skerrett (1999), Frans *et al*., (1999).

Some grass species when grown in nutrient cultures containing NaCl as a sole osmoticum, frequently displayed leaf blade deformation and necrosis as characteristics of severe Ca-deficiency (Kawasaki and Moritsugu, 1979). Physiologically Ca^{2+} stabilizes the cell wall structure to maintain membrane integrity and regulates selectivity of ion transport processes (Hanson, 1983). Membrane permeability may increase by Na^+ displaced Ca^{2+} ions that are associated with the external surface of the plasmalemma (Cramer *et al*., 1985). A significant increase in the Ca^{2+} concentration in the shoots of fodderbeet was observed at day 28 and 35 in the presence of NaCl (Chapter 4, Table 12), which may form part of a highly salt tolerant behaviour of the plants. The magnesium ion concentrations in *Beta vulgaris* subsp. *vulgaris* cv. Majoral did not reduce with the age of the plant, however an increase in Mg^{2+} was observed at 200 mM NaCl in *Beta vulgaris* subsp. *maritima.* The Na^+ concentration in the roots and Mg^{2+} concentration in the shoots showed a significantly negative correlation (r^2 = -0.77) in *Beta vulgaris* subsp. *vulgaris* cv. Majoral, while the same correlation was highly positive (r^2 = 0.91) in *Beta vulgaris* subsp. *maritima*. An increase of Ca^{2+} in the leaves may result in a marked reduction in leaf Mg^{2+} (Bernstein and Hayward, 1958). This Ca^{2+} - Mg^{2+} antagonism could disturb the process of photosynthesis. Reduced photosynthesis was noted in maize due to Mg^{2+} deficiencies (Peaslee and Moos, 1966). High Ca^{2+} concentration in the leaves may also interfere with CO_2 fixation by inhibition of stroma enzymes especially those that are Mg activated (Charles and Halliwell, 1980). There was no disturbance observed in the Ca^{2+} - Mg^{2+} balance and Mg^{2+} tended to increase with a simultaneous increase in Ca^{2+} concentration. This may result in better net photosynthesis of fodderbeet and seabeet in the presence of high NaCl (Niazi *et al*., 1999). This may be one of the explanations for the the salt tolerance of the fodderbeet and seabeet. Similar results were also presented by Knight *et al*. (1997), Davenport *et al*. (1997), Liu and Zhu (1997); 1998), Kinraide (1998) and Bressan *et al*. (1998).

The chlorophyll concentration in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) remained unaffected by salinity, while in seabeet (*Beta vulgaris* subsp. *maritima*) the chlorophyll 'a' and 'b' concentration was significantly reduced by salinity (Chapter 4, Table 14). A significant increase in biomass production could be related to the number of leaves and average leaf area per plant. Both the above-mentioned growth characteristics may also relate to maximum light energy interception by the plants (Niazi *et al*., 2000). The larger leaf area per plant may have supported greater light energy interception in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) than that of seabeet (*Beta vulgaris* subsp. *maritima*). The photosynthetic activity of the plants controls growth and especially dry matter accumulation. One of the roles of photosynthesis is to accumulate plant structural and storage material. The products of photosynthesis may also be important for osmoregulation. Lower water-soluble sugar concentration in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) as compared to sugarbeet has been reported by Quin *et al*. (1980). The sugar concentration increased in both plants species with increasing salinity (Chapter 4, Table 15). This may be related to the increased Mg^{2+} concentration (Niazi *et al*., 1999), because of the importance of Mg^{2+} as a co-factor in almost all enzyme-activated phosphorylating processes. Magnesium is also required for a significant increase in biomass production. The sugar concentrations also increased after addition of Cl^- along with root fresh and dry weights. An increase in the K^+ uptake was reported by Niazi *et al*. (2004) which may increase the translocation of photoassimilates from the leaves to the roots, thus increasing the amount of root dry matter (Hartt, 1970; Magat and Goh, 1988). There was no change in K^+ concentrations noted in the present study. Increases in root yield may be related to the nitrogen content leading to depressed sucrose concentration in the roots (Anderson and Peterson, 1988). In the present study a nitrogenous fertilizer was not added to the pots. Therefore, an increase in sugar concentration may relate to increased uptake of Mg^{2+} in seabeet (*Beta vulgaris* subsp. *maritima*) under increasing salinity.

Translocation of proteins to the shoots may have resulted in a decrease in the total protein concentrations of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) in the later stages of plant growth. This is confirmed by a consequent increase of protein in the shoots. A significant increase in the protein concentrations of the seabeet (*Beta vulgaris* subsp. *maritima*) shoot was observed under increasing salinity in our study (Chapter 4, Table 16). An increase in the sugarbeet protein content due to increased micronutrient nutrition has been reported by Firgany *et al*. (1981); Draycott and Bugg, (1982). Similar results about increases in protein content in leaves under salinity have been reported by Haeder and Mengel (1972). The results of the present study also confirm that total protein content increased under saline conditions.

Compatible osmotic solutes

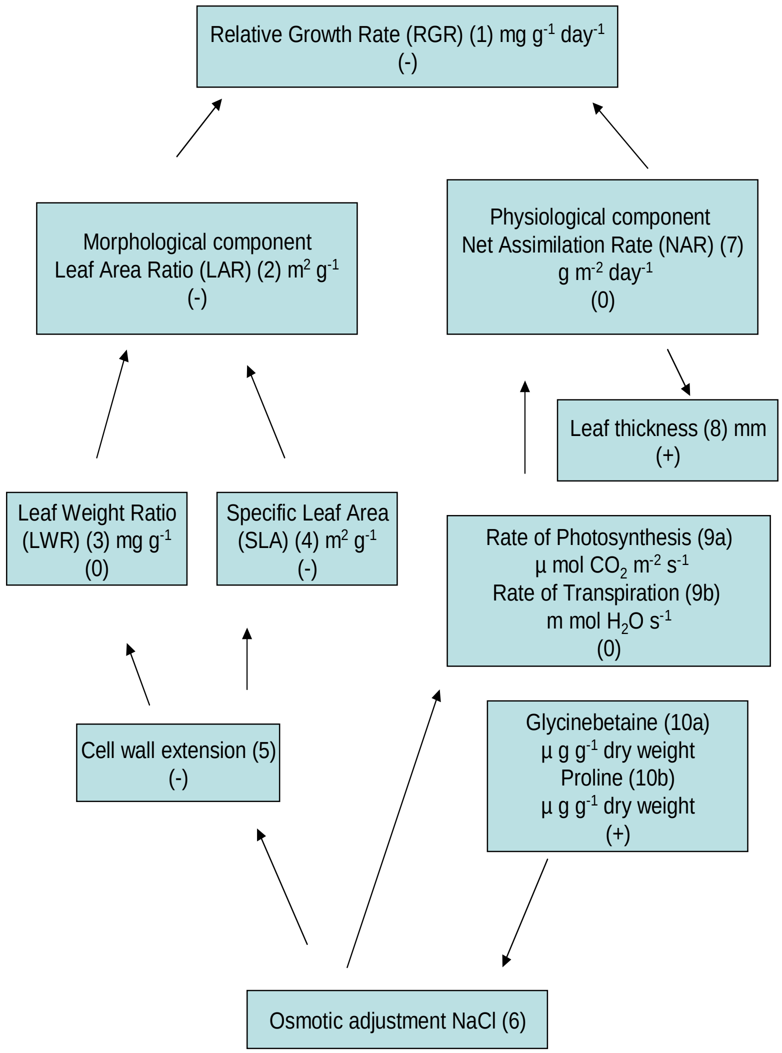
Wyn-Jones and Storey (1981) reported that the accumulation of glycinebetaine is a good indicator of salt tolerance in different plants. Glycinebetaine is very mobile (Mc-Donnel and Wyn-Jones, 1988). Betaine increased with increasing K^+ concentrations in the storage roots of sugarbeet (Beringer *et al*., 1986). A higher glycinebetaine level was also observed in our study (Chapter 4, Table 17). The mechanism of salt tolerance may in part be achieved through accumulation of more K^+, Ca^{2+} and Mg^{2+} ions. Sequestration of Na^+ ions into the cell vacuole is one of the mechanisms to confer Na^+ tolerance to *Arabidopsis thaliana*. The genes involved in this process have been identified as low affinity electro neutral Na^+/H^+ exchangers (Darley *et al*., 2000). Several proteins have been characterized that play a prominent role in the regulation of K^+ and /or Na^+ fluxes (Maathuis and Amtmann, 1999). These ions may help to improve the synthesis of sucrose and protein, which activate the biosynthesis of glycinebetaine, thus increasing the salt tolerance of plants. Seabeet (*Beta vulgaris* subsp. *maritima*) is a halophyte found in saline coastal areas. The aerial part of seabeet (*Beta vulgaris* subsp. *maritima*) is extensively branched and the number of leaves exceed that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). Leaf area per plant of seabeet (*Beta vulgaris* subsp. *maritima*) is less than that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) is the domesticated cultivar originating from seabeet (*Beta vulgaris* subsp. *maritima*) with a larger leaf area per plant, which helps the plant in the interception of sunlight. This will account for the accumulation of a higher concentration of sugar and glycinebetaine which in turn results in higher energy storage by the plants. In the seabeet (*Beta vulgaris* subsp. *maritima*), the higher chlorophyll concentration could not contribute to the accumulation of plant biomass effectively because of the smaller leaf area compared to that of fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). The larger leaves in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) may have successfully contributed to increased carbohydrate content by intercepting more sunlight. Consequently, this may have provided extra energy to overcome salt stress experienced by the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral.

<u>Salt tolerance of fodderbeet, growth analysis, morphological and physiological components</u>

Dry weight per plant in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* did not increase with increasing salinity (200 and 400 mM NaCl) compared to control (0 mM NaCl) (Chapter 4, Fig 1). This indicates that the plant may have adjusted to increasing salinity during the first two weeks of growth. During the later developmental stages the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* decreased significantly with increasing salinity (200 and 400 mM NaCl). The dry weight accumulation per plant was however greater in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral compared to seabeet *Beta vulgaris* subsp. *maritima.* The average leaf area per plant in *Beta vulgaris* subsp. *vulgaris* cv. Majoral was significantly higher than that of *Beta vulgaris* subsp. *maritima*. The leaf area significantly decreased with increasing salinity (Chapter 4, Fig 3). The number of leaves (Chapter 4, Fig 2) and their arrangement in the two cultivars was different. *Beta vulgaris* subsp. *vulgaris* cv. Majoral had a lower number of leaves, with larger individual leaf area per plant and reduced stem length, while seabeet *Beta vulgaris* subsp. *maritima* had a larger leaf number with a smaller individual leaf area and a rosette arrangement of leaves. This may reflect a difference in radiation interception resulting in different dry weight accumulation (Martin, 1986).

The relative growth rate was lower at elevated salinity (400 mM NaCl) in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima*) (Chapter 4, Table 18). After two weeks a decrease in RGR may be related to a significant decrease in leaf area per plant with increasing salinity during the same growth period in fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima)*. It has confirmed the results obtained in chapters 3, 4 and 5 (Fig. 1). Net assimilation rate increased or decreased relating to the RGR of respective species. However, there was no significant difference in NAR after two weeks of growth in both fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral) and seabeet (*Beta vulgaris* subsp. *maritima).* There was no significant decrease in LAR and SLA under saline conditions (200 mM NaCl) for seabeet *Beta vulgaris* subsp. *maritima* (Chapter 4, Table 18) which may be related to an increase in leaf thickness (Niazi *et al*., 1995). It may reflect the increased accumulation of shoot dry matter of seabeet *Beta vulgaris* subsp. *maritima* under increasing salinity. There was no change in LAR which may be related to greater leaf size in *Beta vulgaris* subsp. *vulgaris* cv. Majoral.

Figure 1. Interrelationships of Fodderbeet (*Beta vulgaris* subsp. *vulgaris* cultivars*) and* Seabeet *(Beta vulgaris* subsp*. maritima*) plant growth parameters assessed in chapters 3, 4 and 5 [(0) No Change, (+) Increase, (-) decrease with increasing salinity i.e. 400 mM NaCl]



The leaf area per plant of *Beta vulgaris* subsp. *maritima* was smaller than that of *Beta vulgaris* subsp. *vulgaris* cv. Majoral (Chapter 4, Fig 3). A significantly higher number of leaves in *Beta vulgaris* subsp. *maritima* with smaller leaf area than in *Beta vulgaris* subsp. *vulgaris* cv. Majoral may play an important role in the difference in leaf thickness. The presence of a higher number of leaves per plant leads to a specific pattern for expansion in which the size of successive leaves decreases (Milford *et al*., 1985). The area of the individual leaves in *Beta vulgaris* subsp. *maritima* was relatively small. Leaves of *Beta vulgaris* subsp. *maritima* were fleshy compared to those of *Beta vulgaris* subsp. *vulgaris* cv. Majoral at 400 mM NaCl (Niazi *et al*., 1995). The specific leaf area of *Beta vulgaris* subsp. *maritima* was less at higher salinities (200 and 400 mM NaCl) in the second week than that of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral when compared at the respective harvests. This supports the findings that *Beta vulgaris* subsp. *maritima* tend to have succulent leaves with a higher number of mesophyll tissue layers and intercepted more light energy. Therefore, NAR of the plant did not decrease with increasing salinity up to 200 mM NaCl (Chapter 4, Table 2). Hence the adjustment of the seabeet *Beta vulgaris* subsp. *maritima* to salinity may be related to the activity of the physiological component of plant growth (NAR) under saline conditions. The *Beta vulgaris* subsp. *vulgaris* cv. Majoral had a higher leaf dry weight because of its greater average leaf area per plant, which may relate to a greater LAR. The increase in the LWR with high salinity after one week of plant growth in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral may relate to the improvement in the morphological component of growth for salt tolerance to plant.

The effect of increasing salinity is best observed in solution culture because of a direct association of the roots with the growth medium. Furthermore, the concentration of the salt solution is uniform without any fluctuation in the relevant salinity treatment. In the case of pot-culture studies, although the experiments were conducted under controlled conditions e.g. humidity, light, temperature etc. the salinity fluctuated with the irrigation of the pots. Therefore, the effect of salinity was reduced and plants got temporary relief due to dilution during irrigation. On the other hand, variability in the environmental conditions in the field experiments associated with patchy salinity. The number of irrigations was limited and the salinity/sodicity comprised of a mixture of salts. As a result, the effect of salinity was not uniform on all plants, as in the pot experiments.

Salt affected soils and biosaline agriculture

The saline and saline-sodic soils are mostly calcareous in Pakistan. The release of protons during the exchange of Na^+ with Ca^{2+} on the soil complex may be one of the reasons for the lower soil pH after harvesting. Therefore, regular cultivation of fodderbeet may support further improvement of the soil. The reduction in EC_e of the soil surface may indicate removal of soluble salts by the plants (Chapter 5, Table 4). Magat and Goh (1988) recommended NaCl as fertilizer for production of fodderbeet grown in some soils of New Zealand, while applications of various doses of K (0-60 mg 100 g^{-1} soil) did not improve the leaf and root growth of sugarbeet grown in alluvial soil (Beringer *et al*., 1986), which indicates a limited K^+ requirement of sugarbeet. The comparison of the plant growth of fodderbeet in non-saline and saline-sodic soils at D.I. Khan had shown significantly higher fresh weights of leaves in the four cultivars of *Beta vulgaris* subsp. *vulgaris* under saline-sodic soils. There was variation in the fresh weight of beet among the cultivars. However, fresh weight of beet in cultivars Monored and Polygroeningia was significantly higher when compared with the growth under non-saline soils (Chapter 5, table 5). It proves that fodderbeet required higher salt concentrations for its better plant growth.

The chlorophyll ‘a’ and ‘b’ concentrations were increased significantly under saline conditions (Chapter 5, Table 6). An increase in chlorophyll ‘a’ concentration under saline conditions was significant in *Beta vulgaris* subsp. *vulgaris* cvs. Monoval, Polygroeningia and Majoral. The chlorophyll a/b ratio revealed that chlorophyll ‘b’ increased under saline conditions in all the cultivars except in *Beta vulgaris* subsp. *vulgaris* cv. Monored. This could be a reason due to which dark green leaves were produced under saline conditions. Papp *et al.* (1983) have reported a decrease in the chlorophyll content under saline conditions in sugarbeet. A significant increase in chlorophyll ‘a’ and ‘b’ concentrations and an increased photosynthetic rate may have considerably improved fresh weight of the plant (Chapter 5, Table 5). Products of photosynthesis may also be important for osmoregulation and may help to evaluate the degree of salt tolerance of the plant. Several physiological studies suggested that under stress conditions, accumulation of sugars may occur to varying degrees in different plant species (Taji *et al*., 2002). An increase in chlorophyll ‘a’ concentration under saline conditions was highly significant in *Beta vulgaris* subsp. *vulgaris* cvs. Monoval, Polygroeningia and Majoral. The higher protein concentration of the leaves (Chapter 5, Table 8) and the proline concentrations of beets and leaves significantly increased due to salinity (Chapter 5, Table 9), which may indicate improvement in physiological processes related to salt tolerance of fodderbeet. A significantly higher chlorophyll conctentration of fodderbeet reflects an adaptation under saline-sodic conditions not present in oat and barley (Chapter 5, Table 10). Barley and oat crops have a lower salinity tolerance compared to fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral. Significantly higher chlorophyll concentrations of fodderbeet reflects an adaptation under saline-sodic conditions as compared to oat and barley (Chapter 5, Table 10). Barley and oat have a lower salinity tolerance compared to fodderbeet (*Beta vulgaris* subsp. *vulgaris* cv. Majoral). Introduction of fodderbeet in the salt-affected areas may improve the fodder production for cattle. Mixing of chopped fodderbeet (leaves and roots) with conventional fodder (barley and oat) proved palatable for the cows. The fodderbeet seed production in Pakistan was not successful in the past, which was a constraint in the promotion of fodderbeet cultivation in salt-affected areas. Import of fodderbeet seeds was uneconomic. Recently, a successful attempt has been made to produce fodderbeet seed at an Agricultural Research Station in Swat Valley of Pakistan. In this way the promotion of fodderbeet cultivation has been accelerated through the Deapartment of Agriculture Extension.

CHAPTER 7

Chapter 7

Summary

In the experiments described in this thesis on fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* grown under saline conditions the seed germination of four fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars was first studied in response to salinity (EC 4-20 dS m^{-1}~ 40-200 mM NaCl) and three temperatures (20^o, 25^o and 30 ^oC). The germination rate of seeds of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars at 25^o C was somewhat higher than at 20^o and 30 ^oC. Overall the germination of the fodderbeet seeds decreased with increasing salinity. The germination of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Monoval compared to the *Beta vulgaris* subsp. *vulgaris* cvs. Majoral, Monored and Polygroeningia were somewhat higher at increasing salinity level (chapter 2). Yet at the average salinity (EC 5-15 dS m^{-1}) of saline arable soil in Pakistan, the germination rate was about 70 %.

In growth experiments with increasing salinity (chapter 3 and 4), fodderbeet cultivars were screened for salt tolerance. Fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral showed to be a relatively salt tolerant cultivar. Growth and physiology of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was compared with that of its primary ancestor seabeet (*Beta vulgaris* subsp. *maritima*). Physiological and morphological aspects of growth were studied first in response to salinity in nutrient solution (chapter 3). Both fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* tolerated a salinity level of 200 mM NaCl. Among the four fodderbeet cultivars tested, *Beta vulgaris* subsp. *vulgaris* cv. Majoral was found to be a promising non-conventional fodder crop plant for cultivation in saline arable lands in Pakistan. Further investigations into the growth and

physiology of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* plants were conducted in relation to ion uptake and to the concentrations of osmolytes (sugars, proteins, proline and glycinebetaine) in the shoot tissue under saline conditions (chapter 4). The levels of the osmolytes glycinebetaine and proline in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral and seabeet *Beta vulgaris* subsp. *maritima* increased at intermediate salinity levels. The concentrations of proline and glycinebetaine in fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral tissue are discussed in relation to tolerance to saline conditions (chapter 4).

Another experiment was conducted to explore the growth response of the four cultivars of fodderbeet under saline-sodic field conditions in Pakistan (chapter 5). The plant biomass (beet and leaf) of fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars Majoral and Monored increased when grown under saline-sodic soil conditions compared to non-saline soil conditions. The pH and EC of saline soil decreased (pH 8.15 → 7.56 at 0-15 cm and 8.26 → 8.03 at 15-30 cm soil depth and EC_e 11.38 → 7.39 dS m^{-1} at 0-15 cm and 6.72→5.61 dS m^{-1} at 15-30 cm respectively) after harvesting of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral crop compared to pre-sowing values. The leaf chlorophyll ‘a’ and chlorophyll ‘b’ concentrations increased with increasing soil salinity. The sugar concentrations of the beets of all four fodderbeet cultivars increased with increasing NaCl, while that of the leaf decreased in *Beta vulgaris* subsp. *vulgaris* cvs. Majoral and Monoval. The leaf proline concentrations however, increased under saline conditions in all the cultivars tested indicating fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral to be a salt tolerant plant.

A comparison was made between fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral biomass production with that of two conventional crops, barley and oat, cultivated under field conditions in a saline-sodic soil in Pakistan. Biomass production of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was significantly higher than that of barley and oat. The fodderbeet had a larger number of leaves per plant with a nearly double leaf area per plant compared to barley and oat. Fodderbeet had a three times higher average fresh weight of the whole plant.

FYM is used as a source of nutrients to plants when added to the arable fields. The FYM is used by the farmers to increase the soil fertility status to enhance crop production. Addition of FYM highlighted the economical utilization of otherwise unproductive salt-affected soil in Pakistan by growing fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plants. The mixing of FYM with non-saline soils significantly improved the fertility of the soil. The mixing of FYM to the saline-sodic soils however, depressed the growth of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral plant compared to that grown in soil without the addition of FYM (Chapter 5, Table 12). The increased concentrations of glycinebetaine, sugars and proline of the above- ground parts of fodderbeet may relate to its tolerance of saline-sodic conditions. However, the salt tolerance of fodderbeet in saline-sodic soil was not increased after addition of FYM. The fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral fed to cattle at NARC, Islamabad, Pakistan was found palatable, when the leaves and beets were chopped and mixed with the conventional fodder (barley). There was no disorder in the digestion of the cows during the 15 days feeding of fodderbeet. The results of these experiments are discussed in chapter 5.

The experiments conducted in the laboratory, greenhouse and field (chapter 2, 3, 4, and 5) have revealed that the fodderbeet cultivar *Beta vulgaris* subsp.

vulgaris cv. Majoral can tolerate a salinity of 200 mM NaCl in the root medium. The production of biomass (leaves and beet) of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral was shown to be comparatively higher than that of two conventional fodder crops, barley and oat grown in Pakistan. The day temperature during the winter season in Pakistan ranges from 20^o to 30 ^oC, which is suitable for the germination of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral seeds based on the results of the germination experiment described in chapter 2. Therefore, it may be expected that fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral could be economically cultivated on saline arable land in Pakistan as a fodder crop in addition to the conventional crops, barley (*Hordeum vulgare*) and oat (*Avena fatua*). The cropping season of fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral in Pakistan (mid October to mid April) also coincides with the period when there is a shortage of conventional fodder for cattle. Introduction of the fodderbeet *Beta vulgaris* subsp. *vulgaris* cv. Majoral will help in the utilization of non-productive saline-sodic soils by production of a fodder crop during this period of shortage of fodder for cattle.

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CURRICULUM VITAE

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Studies

Affiliation	Peshawar University, Peshawar, N.W.F.P., Pakistan
Dates	August, 1971- September, 1973
Subject	Studied Botany, special interest in medicinal plants
Affiliation	Quaid-e-Azam University, Islamabad, Pakistan
Dates	May, 1979 – October, 1981
Subject	M. Phil. research: Studied Plant Ecology, special interest in salt tolerance of plants. Thesis on Effect of NaCl and Zn on tomato.
Affiliation	Vrije Universiteit, Amsterdam, The Netherlands.
Dates	Since February, 1990
Subject	Ph. D research: Salt tolerance of halophytes.

	Thesis on: The response of fodderbeet to salinity: Introduction of a non-conventional fodder crop (fodderbeet) to salt affected lands of Pakistan
Employment	**Lecturer** in Botany Department of Education, Government of Azad Kashmir, Pakistan, January 1974–September 1979
	Research fellow, Department of Biological Sciences, Quaid-e-Azam University, Islamabad, Pakistan, September, 1979-November, 1981
	Scientific officer: November, 1981–December, 1989.
	Senior Scientific Officer: January, 1990–August, 2005,
	Principal Scientific Officer: since August, 2005 todate.
Projects completed	Saline Agriculture Project: 1981-1985
	Development project on Plant Stress Physiology: 1985- 1991
	Application of gypsum to improve fertility of saline-sodic soils: 1995-1999
	Agricultural linkages Project on Management of salt-affected soil and brackish waters in Pakistan: since 2003-2006
Honorary assignment	Editor, Pakistan Journal of Soil Science: since 2004 todate

LIST OF PUBLICATIONS

I. Refereed Journals of International Repute:

Niazi, B. H., Rozema, J., Salim, M. and Tariq, A. 2005. Effect of Pre-treatment and Post-treatment of growth hormones (Kinetine and Abscisic Acid) on ion concentration and biochemical contents of fodderbeet and seabeet under saline soil conditions. Gen. App. Plant Physiol. 31(1-2): 89-104.

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Acknowledgements

Scientific workshops and conferences provide opportunities to members of the scientific community to introduce themselves. Sometimes a HELLO leads to good relations and interactions in scientific research being conducted in different countries. It also happened to me when I met Dr. Jelte Rozema in an International Biosaline Research Workshop held September 1985 at Karachi, Pakistan. Credit goes to Dr. Zaib who was the anchor person in this introduction. At that time Dr. Jelte Rozema visited the salt-affected area in the Sind and Punjab provinces of Pakistan. His vast experience in the problematic lands affected with high salinity brought an idea of introducing the salt tolerant fodderbeet species into these degraded arable lands.

The cultivation of salt tolerant fodder crop (Fodderbeet *Beta vulgaris* subsp. *vulgaris* cultivars) has since proved its suitability in the agro-ecological zones of two provinces i.e. Punjab and N.W.F.P. It was the beginning of cooperation between the Vrije Universiteit Amsterdam, The Netherlands and NARC, Islamabad, Pakistan and I happened to be the beneficiary. To achieve the skills and learn advanced techniques used to evaluate the salinity tolerance of plants species, a visit was organized which was funded by Nuffic.

During my stay at the VU (February, 1990 to April, 1991), I had the wonderful experience of cooperation and earned love from my colleagues working in the Department of Ecology and Ecotoxicology, Faculty of Biological Sciences. I will not forget lessons received from Dr. Rinus Otte for learning of computer operation and the use of statistical packages for analysis of scientific data obtained from research studies. The guidance of Dr. Ger Lenssen for growth analysis using spread sheets developed by him, still helps me and my colleagues at NARC. I am highly obliged to Dr. Peter Leendertse, Dr. Hans Linders, Dr. Ric de Vos and other research fellows for their sincere help. All the members of the Plant Ecology group helped me in one way or the other, which I think is a great asset to me. I must acknowledge the hands on training on various instruments by Rob Broekman which enabled me to analyze the data for completion of this thesis. How can I forget Martin Stroetenga, who recognized me even after 15 years when I visited VU again in October, 2005 by saying “Hi Niazi, it is you. You are still young” (It sounded very sweet to me and I felt, it is not too late to get “promotie” at this moment. I am also obliged to my friend Faqir Muhammad, who gave me company and made me feeling at home by offering unplanned visits to his residence in a typical Pakistan style. The visit for the Ph D ceremony is also being hosted by him.

At the end of stay at VU, the results obtained from the well planned studies by Dr. Jelte Rozema encouraged us and we decided to continue our work in Pakistan. With the kind consent of Prof. Dr. W.H.O. Ernst we also agreed upon to compile the data based on the studies conducted at the VU and NARC, to prepare scientific papers and eventually a Ph. D. thesis. The cooperation of Dr. A.H. de Boer (co-promoter at the VU) for sparing his precious time to add valuable scientific and technical suggestions in the improvement of the manuscript up to “VU standard” is highly acknowledged.

In Pakistan, Dr. M. Salim (co-promoter in Pakistan) very happily approved the Ph. D. plan and my colleagues offered their sincere help in planning and conducting the research studies. I acknowledge the cooperation of Mr. Badr-uz-Zaman, Dr. M. Yasin, Dr. Rahmatullah, Dr. Manzoor Ahmad and other co-authors of our publications related to promotion of fodderbeet cultivation in salt-affected soils in Pakistan. The visit of M. Sc. students from the Vrije

Universiteit played a vital role in the cooperation between the research organizations of two countries. The visit conducted by Mr. Eric Naus, Mr. Ronald Huisman, Mr. Hado Steen and Mr. Marcel Schoehuys to NARC, Islamabad, Pakistan and their research done on fodderbeet strengthened the data obtained and paved way for planning further studies. Their role in the promotion of cooperative research in this field is highly appreciated.

The author is highly obliged to the reviewers of the thesis for their valuable comments. The author acknowledges the favours they have given in reviewing the thesis. The funding by the Rutgerfonds, Vrije University for my visit to the Department of Systems Ecology in 2005 and 2007 to complete my thesis under the supervision of Prof. Dr. Jelte Rozema is highly appreciated and acknowledged. I also appreciate the cooperation of Joordens Zaden BV, Kessel, The Netherlands for providing fodderbeet seed and their interest in the cultivation of fodderbeet in Asian countries. I am indebted to my colleagues, Professor Dr. Marinus L. Otte, Chair, Department of Biological Sciences, North Dakota State University, Fargo, USA and Dr. Athar Tariq, California Department of Food and Agriculture, Sacramento, USA for their inputs in improving the thesis.

Last but not least, I am highly obliged to my family who very patiently waited for the completion of this thesis. Their encouraging behaviour for reinforcement of the once slowed down progress on the “promotie” project of thesis would never be forgotten.

I hope that the cooperation in the research between the Vrije Universiteit and NARC would be sustainable.

Banaras H. Niazi